Blood and Vascular Targets for Magnetic Field Dosing

Harvey N. Mayrovitz, PhD

1.0 INTRODUCTION

1.1 Dose as a Factor in the Early Days

Horatio Donkin, a senior assistant-physician to East London Hospital for Children at the time, writing in the British Medical Journal in 1878 (Donkin 1878) provides a number of remarks on magnetic therapeutics. These remarks were chiefly in response to claims made regarding magnet use by an English professor Gamgee (Gamgee 1878) of the ability of metals and magnets to alter various neurological functions described by the famous French physician M. Charcot and others of the time. Among several targeted critiques offered by Donkin the one that captures the essence of the issue was put by him in part as follows. "……. *the physiologist, being somewhat conversant with the infinite permutations and combinations of nervous and so called "functional" phenomena in the human organism, is, as a rule, aware of the complexity of the matter in hand, when these phenomena become the subject of a scientific investigation, and recognises, as physicians strive to do, the necessity of applying the most rigorous methods of research before arriving at any conclusion in so difficult a field of observation"*. In the same year (1878), in the 3rd edition of his text "Medical Electricity", Roberts Bartholow (Bartholow 1887) wrote about the "Physiological effects of magnetic applications" in which he takes the view that with respect to the application of a magnet to the skin "*The production of physiological effects, that can be recognized, is merely a matter of magnetic intensity*" or as we would state – a matter of dose – with the intrinsic – but unsubstantiated thought that bigger was better. In 1892, F. Peterson a physician and A.E. Kennelly an engineer combined their efforts to test this concept with experiments done at the Edison Laboratory in Orange N.J. (Peterson and Kennelly 1892). They built a large iron electromagnet, reported to need two strong men to lift, that produced field strength of 5000 G within the confines of its poles separated by 1.2 cm. Introduction of various substances within the pole-space and the observation of the effects was the mode of investigation used. Human and frog blood placed on a slide within the pole-space and observed microscopically *"failed to show the feeblest traces of polarization, movement or vibration"*. Similar observations of the frog's web blood circulation at field strength of 1500 G also showed no visible effect of the field. Contrastingly when a 1-2 mA current was passed from toe to ankle the circulation was arrested and restored when the current was removed. Based upon these and a series of other experiments the authors *concluded "…… that neither direct nor reversed magnetism exerts any perceptible influence upon the iron contained in the*

blood, upon the circulation, upon ….". It was their view that *"The ordinary magnets used in medicine have a purely suggestive or psychic effect, and would in all probability be quite as useful if made of wood",* a view that is echoed by many today. Despite this they had an open mind as evidenced by the close of their conclusion by stating that *"… we do not deny the possibility of there being invented someday magnets enormously more powerful than any yet known to us, which may produce effects upon the nervous system"* this being a clear foreshadowing of the potential importance of dose at the target site as a factor. Indeed others of the time had put forward the use of what we would call double-masked experiments showed that magnets so applied could alter skin temperature and muscular contraction (Columbia and Richet 1880).

1.2 Blood and Vascular Targets

When considering possible blood and vascular impacts of electromagnetic field (EMF) stimulation, as is the main topic herein, there is good reason to believe that effects on cellular membranes and associated ion transport are involved since it is these processes that ultimately regulate function. The possibility of membrane transport modulation that is dependent on frequency, amplitude or duration "windows" makes the search for such EMF-membrane transport interactions elusive but has been considered by several investigators (Berg 1999). To provide a suitable and manageable template for discussion it is our plan to mainly discuss blood and vascular targets that relate to potential EMF impacts on blood flow with the components of **figure 1** useful to illustrate some major target components under consideration. When considering possible dose-dependent aspects (Markov and Colbert 2000, Colbert, Markov et al. 2008) it should be recognized that for static magnetic fields (SMF) dose mainly relates to field intensity effects at the target whereas for pulsed electromagnetic fields (PEMF) it also includes frequency parameters and induced currents. Thus we have chosen to consider and discuss separately the SMF and PEMF effects on targets that impact blood flow. Further to help provide a contextual basis, brief overviews of some physiological basis and blood flow control involvement are provided as appropriate for which **figure 1A** serves well as an initial canvas.

Aside from EMF-related forces that act on red blood cells (RBC) that are discussed in section 3.1, blood velocity (cm/sec) and flow (ml/sec) changes are expected to result from EMF actions that cause changes in blood vessel diameters (D) since single vessel vascular resistance depends on $1/D^4$.

Physiologically, changes in diameter occur when ion channels within the membranes of vascular smooth muscle (VSM) or endothelial cells (EC) change state to allow more or less entry or exit of selective ions that control the relaxation state of the VSM. Often this state change depends on the level of the transmembrane potential which can "gate" open more or close more selective channels. Such interactions may involve a host of intermediary components and processes but one important one

is the flux of Ca^{++} through selective ion channels in which an inward Ca^{++} flux is facilitated by a partial depolarization of the membrane potential. When this occurs in VSM the result is an increase in VSM contraction force reducing the vessel's diameter (vasoconstriction) as simply schematized in **figure 1B**. Physiologically these events can be caused by changes in the impulse rate of the nerves that innervate the blood vessel walls since the amount of vasoactive substance released by the nerve terminals, such as norepinephrine (NE in figure 1B), depend on the impulse rate. Contrastingly if Ca^{++} influx is increased in endothelial cells, biochemical processes are triggered that include more release of nitric oxide (NO) that diffuses to VSM causing these cells to be less contracted (more relaxed) that generally results in an increase in the vessel's diameter (vasodilation) as schematized in **figure 1C**. This EC initiated effect depends on the EC synthesis and release of vasoactive substances such as nitric oxide (NO). NO release is induced by chemical and physical stimuli including acetylcholine (Ach) and adenosine triphosphate (ATP) and mechanical events such as wall shear stress. NO is synthesized from L-arginine in the presence of constitutive nitric oxide synthase (cNOS) that is Ca^{++} dependent and inducible nitric oxide synthase (iNOS) that is dependent on the presence of cytokines and other substances. NO diffuses to VSM where it activates cytoplasmic guanosine tripohsphate (GTP) that then increases cyclic guanosine monophosphate [cGMP], causing a decrease in Ca^{++} entry and a decrease in cytosolic free Ca^{++} in VSM resulting in relaxation.

2.0 BLOOD FLOW MODULATION BY EMF: GENERAL CONSIDERATIONS

Blood flow changes associated with EMF stimulation may occur in two generalized ways, one is related to potential blood property changes and the other to vascular and hemodynamic changes.

2.1 Blood Property Change

These would include modification of blood's properties that subsequently decrease or increase blood flow under the same hemodynamic driving forces. Examples of such changes would be alterations in blood viscosity, red blood cell (RBC) orientation or RBC physical or chemical properties.

2.2 Vascular and Hemodynamic Changes

These would modification of blood vessel diameters and other properties that either directly affect blood flow via alterations in vascular resistance to blood flow and/or cause changes in blood pressures that directly affect blood flow. With reference to **figure 1A**, examples include changes in vascular smooth muscle (VSM) membrane potentials that cause alterations in channel ionic currents (e.g. Ca^{++}) that result in vessel diameter (D) changes. Increased amounts of Ca^{++} entry cause vasoconstriction. Another example would be EMF-related changes of nerve membrane potentials that cause changes in vasoactive substance release such as norepinephrine (NE) from thereby affecting vessel diameter and blood flow. A third example would be EMF-related changes in endothelial cell

(EC) membrane potentials and currents that would alter their release of vasoactive compounds such as the vasodilator nitric oxide (NO) and the vasoconstrictor endothelin (ET). These processes in part depend on Ca^{++} fluxes in endothelial cells as discussed in section 1.2.

2.3 Magnitude of Blood Flow Change

In assessing potential cellular or vascular EMF-related effects on blood flow a key question is if and how much change (increase or decrease) occurs with an applied SMF or PEMF. It is not useful in our opinion to consider or search for mechanisms or dosing issues in which there first has not been demonstrated such blood flow changes. So our approach is to first examine what is known regarding blood flow changes. Further, because "healthy" or "sick" targets may show different outcomes depending on dose or other factors EMF-related impacts present or not-present in healthy tissue may not apply to "sick" or otherwise traumatized tissues and is a concept that should always be borne in mind.

3.0 BLOOD FLOW MODULATION BY EMF: WHAT DO WE KNOW?

3.1 Direct Force-Related Effects in Blood Vessels

Blood flow is partly the movement of iron-containing RBC and various ions within and together with electrically conducting blood plasma. The electrically conducting blood flowing in a magnetic field experiences an electromotive force that induces currents in the blood. In turn these currents produce a magnetic field that perturbs the originally applied field and also causes an electromotive force that affects the blood flow itself. If the applied field is a SMF applied at right angles to the flow then the effect is reduced blood flow. One physical aspect thought to be causing this is an alteration in the velocity profile caused by the SMF. In earlier theoretical and experimental work the effort was directed to characterizing changes in steady laminar flow for which velocity profiles were caused to shift from parabolic to a more blunt profile (Shercliff 1953). The profile shift and thereby the effect on flow depends on a parameter denoted as $M = \mu Hr(\sigma/\eta)^{1/2}$ in which μ is permeability, H is the applied field, r is half the flow channel width, σ and η are the fluid conductivity and viscosity. Calculations and some experimental data indicate an increase in flow resistance with increasing values of M. The magnitude of this effect has been further explored theoretically and experimentally for a range of SMF doses. Parenthetically, a magnetic field (**B**) applied 90^o to the velocity (**v**) of flowing blood generates an electric field (**E**) that is orthogonal to both **v** and **B** with a value determined as **E** = **v x B**. So potential differences (ϕ) between opposite walls of a blood vessel with diameter D (ϕ = ED) are directly related to **v** and forms a basis of a blood flow measurement (Kolin, Assali et al. 1957). The relationship **v x B** also shows that the blood flow reducing force is

dependent on the magnitude of the blood velocity, which in human is greatest in the aorta. Theoretical analyses using Navier-Stokes equations have predicted a 10% reduction in aortic blood flow due to a 5 T SMF (Chen and Saha 1984) whereas simulated fields in an arterial model (Sud and Sekhon 1989) predicted a magnetic field related reduction in ascending aorta blood flow of 1.3% for a 12.5 T field at the target. Blood flow simulations with 15% saline solutions (Keltner, Roos et al. 1990) exposed to SMF also showed reduced flow, but by less than 1% if exposed to 2.35 T and 2-3% if 4.7 T.

A theoretical analysis that takes into account the non-zero electrical conductivity of vessel walls and tissues (Kinouchi, Yamaguchi et al. 1996) indicates a dose-dependent reduction in aortic blood flow (Q) approximately as the square of the applied magnetic field that, based on analysis of their data, can be represented as %Q reduction $=0.052B^{1.973}$ in which B is in Tesla. It is noteworthy that when wall and tissue conductivity is included in a more complete analysis, the calculated SMF flow reduction is somewhat greater then if these conductivities are considered to be zero. This is in part due to a greater magnetically induced force tending to retard blood flow. Based on some of these findings it appears that only in blood vessels with the highest blood velocities will there be magnetic forces sufficient to reduce blood flow and then only when sufficiently high SMF are applied. Thresholds for 5% and 10% reductions in aortic blood flow appear to be in the range of 10.1 T and 14.4 T respectively (Kinouchi, Yamaguchi et al. 1996) as determined from the above equation.

A component not specifically included in the prior mentioned theoretical models and analyses were RBC magnetic properties. These arise in part due to the hemoglobin-iron-oxygen complex and electronic spin configurations(Nakano, Otsuka et al. 1971) that in the presence of an applied magnetic field (H) result in an induced RBC magnetic dipole per unit volume (m). This is associated with an RBC a magnetic susceptibility ($\chi = H / m$) that is diamagnetic ($\chi < 0$) if hemoglobin is oxygenated and paramagnetic ($\chi > 0$) if deoxygenated. One consequence is that an RBC experiences a force/volume (**F**) that affects its motion in the presence of applied magnetic fields. This force can be expressed in various forms(Watarai and Namba 2002, Bor Fuh, Su et al. 2004, Han and Frazier 2004, Haverkort, Kenjeres et al. 2009) one being $\mathbf{F} = \chi\, \mathbf{H}\, \nabla\mathbf{B}$ in which χ is most accurately expressed as the difference between χ_{RBC} and χ_{PLASMA} (Zborowski, Ostera et al. 2003). If there were spatial variation only in the z-direction then this simplifies to $\mathbf{F} = \chi\, \mathbf{H}\, \partial\mathbf{B}/\partial z = (\chi/\mu)\, \mathbf{B}\, \partial\mathbf{B}/\partial z$. Such magnetically based forces cause RBC to experience a torque ($\boldsymbol{\tau} = \mathbf{m} \times \mathbf{B}$) which tends to align the disk face plane parallel to the applied field direction. This rotational movement results in relative motion between RBC and plasma that causes frictional energy loss and thereby an increase in the effective viscosity of the blood in an apparent dose-dependent manner based on a novel electrohydraulic discharge test (El-Aragi 2013).

These RBC rotational features may not be exclusively dependent on hemoglobin presence or oxygenation state since experiments indicate that RBC devoid of hemoglobin when suspended in solution orient themselves with the plane of their discoid axis aligned with the applied field (Suda and Ueno 1994). The alignment is dose-dependent over reported ranges from 2 to 8 T (Suda and Ueno 1994) and between 1 and 4 T (Higashi, Yamagishi et al. 1993) with the rotational torque accounting for this alignment theorized to be caused by a membrane dependent directional anisotropy of χ that favors the observed orientation (Higashi, Yamagishi et al. 1993, Suda and Ueno 1994, Takeuchi, Mizuno et al. 1995, Higashi, Asjoda et al. 1997). However, as opposed to these effects observed when RBC were suspended in phosphate buffer solutions, when whole blood was used and similar exposed to fields ranging from 2 to 8 T, essentially no orientation with the applied field was observed(Suda and Ueno 1996). Despite these somewhat divergent observations, perhaps partly due to oppositely directed influences of transmembrane proteins and the lipid bilayer (Higashi, Yamagishi et al. 1993), the fact that SMF do increase blood's effective viscosity and decrease blood flow has been experimentally demonstrated(Haik, Pai et al. 2001, Yamamoto, Nagayama et al. 2004, El-Aragi 2013). The viscosity increase is dose-dependent reported as 3.7% for 1.5 T exposure(Yamamoto, Nagayama et al. 2004), about 8% for 3.0 T (Haik, Pai et al. 2001) and near 20% for % 5 T (Haik, Pai et al. 2001). However, the magnetic effect on viscosity, at least at 1.5 T, appears to depend on the shear rate with which the blood is flowing with only a 0.3% increase observed at a shear rate of 130 s^{-1} (Yamamoto, Nagayama et al. 2004) with 2.6 % and 3.7% increases at shear rates of 49.4 and 18.3 s^{-1}. Average shear rate (γ) in a circular blood vessel with laminar steady flow is calculable from average blood velocity (V) divided by the blood vessel diameter (D) as $\gamma = 2V/D$ or in terms of volumetric blood flow (Q) as $\gamma = (8/\pi D^3)$ Q. Thus the magnetic effect depends on both the magnitude of the flow and the diameter of the vessel in which the flow is occurring. Applying this concept to the human vasculature it is the veins that normally experience the lower shear rates and would most likely be vulnerable to the above magnetic effects. Calculations based on measured blood velocities and vessel lumen areas in superior vena cava(Wexler, Bergel et al. 1968), femoral vein(Willenberg, Clemens et al. 2011), inferior vena cava(Wexler, Bergel et al. 1968), jugular and vertebral veins(Ciuti, Righi et al. 2013), and saphenous vein(Abraham, Leftheriotis et al. 1994) reveal the following calculated average shear rates; 8.2, 13.2, 16.5, 35.2, 53.9 and 56.6 s^{-1} respectively. Thus, the low venous shear rates combined with the deoxygenated state of venous blood suggests that the greatest magnetic effect of the type described would be detectible in the post-capillary vasculature. As far as this author knows, this avenue of research has yet to be specifically undertaken.

3.2 Effects in Smaller Blood Vessels of Animal Models

Normal blood flow conditions in smaller blood vessels including the microcirculation differ from that in larger vessels. The net flow direction in a specific artery or vein is generally known. Contrastingly, smaller arterioles, capillaries and venules within tissues and organs tend to exist as networks with variable and possibly near-random orientations among flow directions (Mayrovitz, Wiedeman et al. 1975, Mayrovitz, Wiedeman et al. 1976, Mayrovitz, Kang et al. 1987, Mayrovitz, Moore et al. 1990, Mayrovitz 1992). Thus it is unlikely that magnetically related vector forces as discussed in the previous section would have much of a net impact on flow. However, if applied fields affect blood or tissue components that directly or indirectly alter vascular diameters then the variable directional aspect is no longer very important. So with this in mind we ask two broad questions; 1) what is the specific evidence of EMF related changes in small blood vessels or tissue blood flow and 2) what might be mechanisms involved?

Placement of rats into the bore of a superconducting SMF of 8T for 20 minutes was used to assess pre and post exposure effects on blood velocity in small venules (20-40 μm) of the skin (Ichioka, Iwasaka et al. 1998) via microscopic observations using the skin fold chamber with velocity measured using the dual-slit method(Intaglietta, Silverman et al. 1975) Compared to pre-exposure the 1st minute of post-exposure showed a 17% increase that the author speculated was due to an exposure-related blood flow reduction with a subsequent post-exposure transient hyperemia. The apparent hyperemia lasted about 10 minutes and was not present in sham exposed animals. The interpretation of this data is complicated by the fact that the entire animal was exposed to the field so that the effect if real could be one due to flow reduction in the large vessels with the observation in small venules being a reflection of such an upstream event.

Possibly arguing for a direct effect is the fact that the venules were observed to be dilated after exposure. But even this might be explained as a response to a prior blood flow decrease. Subsequent measurements by these authors (Ichioka, Minegishi et al. 2000) using laser-Doppler methods showed a slight reduction in blood perfusion during the 20 minute exposure but no associated hyperemia thereby leaving the question still open. An associated skin temperature decrease during exposure may be related to factors other than blood flow reduction (Ichioka, Minegishi et al. 2003) and in fact may have been the cause of the small blood flow reduction previously reported (Ichioka, Minegishi et al. 2000). However, later work using a similar dorsal skin fold chamber to observe the skin skeletal muscle microcirculation demonstrated an apparent dose-dependent blood flow effect (Brix, Strieth et al. 2008) when only the chamber and not the whole body was exposed to varying SMF intensities. Analysis of

video recordings of 24 capillaries for each of 10 awake hamsters obtained during SMF exposure showed a dose-dependent decrease in capillary RBC velocity with an apparent threshold for change at near 500 mT at the target. This velocity reduction occurred within one minute of exposure and was associated with no measured change in capillary diameter, functional capillary density or systemic blood pressure. Quantitatively an average pre-exposure capillary blood velocity of about 0.37 mm/s was reduced to 0.22 mm/sec (40%) at a target field intensity of 587 mT. This reduction could be reversed by reducing or eliminating the field intensity. In interpreting these important findings we suggest that the absence of a diameter change would be expected since capillaries do not have VSM. With no capillary diameter change the above velocity change represents a hefty 40% flow reduction. Subsequent measurements of diameters of arterioles feeding the capillary network showed no significant SMF-related change. This finding would seem to negate the possibility that the observed decrease in capillary flow could be attributed to SMF-related arteriolar vasoconstriction. However there is a caveat to this that depends on the accuracy with which such arteriolar diameters are measured given the inverse 4^{th} power dependence of vascular resistance on diameter. Whether the observed reduction is due to SMF-related increases in blood viscosity or orientations as previously described or due to RBC displacement in inhomogeneous fields (Okazaki, Maeda et al. 1987) is as yet unknown. A mathematical model analysis that includes a drag force due to interactions between a central core of flowing RBC and a cell free periphery plasma indicates a dose-dependent effective viscosity increase and corresponding flow decrease in small vessels that increases with increasing field gradient (Bali and Awasthi 2011). This analysis requires a number of assumptions so whatever the mechanism turns out to be it appears to not occur in a measurable way for field intensities less than about 500 mT (Brix, Strieth et al. 2008).

A direct effect of SMF on skin microcirculation might be inferred from studies on rabbit ear microvessel vasomotion (Okano, Gmitrov et al. 1999). The rabbit-ear circulation was exposed to a SMF of about 1 mT for 10 minutes and effects of infused norepinephrine (NE) or acetylcholine (Ach), alone or with the magnetic field present were evaluated. It was found that in the absence of the SMF, NE caused vasoconstriction and Ach caused vasodilation but with the SMF, the prior NE vasoconstriction was blunted or blocked and Ach vasodilation was blunted or blocked. The authors called this a biphasic response. But, it was not possible to assess the direct effect of the SMF on microvascular blood flow without infusing vasoactive substances that modified vascular tone so it is not known if the SMF by itself causes an increase, decrease or no change in blood flow. Without pharmacological modification, a SMF with doses of 1, 5 and 10 mT caused both increased and decreased vasomotion within 10 seconds of SMF activation (Ohkubo and Xu 1997) independent of

dose. Based on these and further experimental findings in animals (Xu, Okano et al. 1998, Ohkubo, Okano et al. 2007) an interpretation is that there may be a SMF induced vasodilation if a high vascular tone is present and vasoconstriction if a low vascular tone is present. An important note on the above observations is that they are based on a measurement that depends on the relative optical density of the microscopic field being viewed. The method, known as microphotoelectric plethysmography (MPPG) (Asano, Yoshida et al. 1965) senses optical density changes of the entire microscopic field being viewed and uses the magnitude of these as an index of vasodilation and the standard deviation of these changes as an index of vasomotion. It does not directly measure blood flow nor is it certain whether the vasodilation is that due to the venous or arteriolar vessels. Application of this method to assess changes in the rabbit ear circulation when exposed to a 0.25 T SMF indicated an increase in MPPG that occurred about 20 minutes after field application(Gmitrov, Ohkubo et al. 2002). That was interpreted as an increase in blood flow. Whether or not these changes actually indicate a blood flow change, the MPPG measurement that was made on tissue exposed to a much higher B-field dose than used in earlier studies (Ohkubo and Xu 1997, Okano, Gmitrov et al. 1999) revealed the absence of a prior reported vasomotion-like feature and a delay of about 20 minutes in the B-field response, further pointing out the relvance of dose in a potential physiological response. Bidirectional changes in arteriolar tone have also been noted to occur almost immediately in rat spinotrapezius microvessels when an exteriorized preparation was exposed to a 70 mT SMF (Morris and Skalak 2005). Further discussions on electromagnetic differential field effects on microcirculation and with respect to wound healing processes have recently become available (Mayrovitz 2015, Ohkubo and Okano 2015).

3.3 Effects on Vascular Cells

Two main vascular cell types to be considered as potential targets for SMF-related blood flow modulation are endothelial cells (EC) and vascular smooth muscle (VSM) cells. As schematized in **figure 1A**, EC pave the inner surface of all blood vessels and are secretors of various vasoactive substances that may cause vasodilation or vasoconstriction. For example vasodilation may be caused by release of nitric oxide (NO) or vasoconstriction caused by the release of endothelin (ET). VSM is present in most blood vessel types and when VSM is caused to contract there is generally a reduction in blood flow whereas if it relaxes vasodilation occurs and blood flow increases. The state of VSM is determined by multiple factors but one of the common denominators is Ca^{++} entry or exit from EC and VSM via ion channels. For example, release of NO from EC and its vasodilatory effects on VSM is mediated by Ca^{++} entry into EC. Conversely, Ca^{++} entry into VSM is associated with increased vasoconstriction and blood flow reduction. In both cell types trans-cellular Ca^{++} flux is largely determined by the cell's membrane potential that in turn controls the ion channel's gate state. Shifts in

the membrane gate-state depend on conformational changes in membrane spanning proteins that require force changes that may in part depend on the interaction between ion drift currents and an applied field (St Pierre and Dobson 2000, Hughes, El Haj et al. 2005) . Given the above factual physiological scenario we may ask the following question. To what extent is there a connection between applied SMF and altered states of either cell type that would suggest an SMF-related blood flow modulation?

3.3.1 Endothelial Cells

Low dose SMF (0.03mT – 0.12 mT) affects proliferation rates of cultured human umbilical vein EC when administered for 24 hours but has no apparent affect on NO concentration. (Martino, Perea et al. 2010, Martino 2011). Pulsed magnetic fields, PMF (15Hz and 1.8 mT) acting on isolated cardiac endothelial cells also indicated increased cell proliferation (Li, Yuan et al. 2015) and SMF may play a role in arteriogenesis (Okano, Tomita et al. 2007, Okano, Tomita et al. 2008) but there appears to be no experimental work directly showing a link between an applied SMF and altered vasoactive substance release from endothelial cells. Given the current availability of a variety of endothelial cell types it would seem that focused experimental work directed towards this area would be useful.

3.3.2 Vascular Smooth Muscle Cells

Virtually no experimental work has been systematically carried out in which the responses of cultured VSM cells to either SMF or PMF have been evaluated and reported. In view of the important role VSM play in the control of blood circulation this appears to be an important target for future research. However, it has been shown that gradient SMF can alter frog sciatic nerve c-fiber conduction in a dose-dependent manner (Okano, Ino et al. 2012) with a slight but significant reduction at an exposure of 0.7 T but not at 0.21 T. If translatable to sympathetic nerves that innervate blood vessels this could imply a potential vascular effect. However, more work in this area is importantly needed. Other vascular effects are potentially subsequent to alterations in circulating vasoactive substances that in turn may impact VSM. An example of this is the decrease circulating levels of angiotensin II and aldosterone of hypertensive rats exposed to whole body SMF. The normal progression of hypertension with age was blunted when they were continuously exposed to SMF of 10 mT and 25 mT apparently due to reduced levels of both hormones (Okano and Ohkubo 2003). Both of these hormones cause vasoconstriction mediated in part through actions on VSM. Additional vascular-related processes appear to be involved when baroreceptors (BR) in the carotid sinus (CS) of rabbits are exposed to 0.35 T SMF. Bilateral CS exposure was associated with a decrease in mean blood pressure and an increase in an index of blood flow in the rabbit ear(Gmitrov and Ohkubo 2002). These changes occurred in the absence and presence of intravenously administered Ca^{++} channel blocker Verapamil. However, an

SMF-induced increase in BR reflex sensitivity present without intervention was absent in the presence of verapamil administration. This suggests that the SMF-effect on blood pressure and BR sensitivity, which is an index of heart rate change caused by pharmacologically induced small blood pressure changes, depend on Ca^{++} flux processes with multiple possibilities for mechanisms and sites of action.

3.4 Effects on Human Circulation

There are two broad aspects to consider when addressing the issue of SMF effects on human circulation. One relates to the exposure of the whole body or specific functional targets such as the carotid sinus area and the other relates to assessing direct effects on circulation of specific tissues such as skin or muscle.

Whole body 30 minute exposure within an MRI in which only the SMF was active (1.5 T) increased nitric oxide (NO) metabolites (Nitrite, NO_2^- + Nitrate NO_3^-) by about 18% in 33 young men with no change when the field was not activated in a separate group (Sirmatel, Sert et al. 2007). The possible significance of this finding goes to the expansive role of NO as a cardiovascular modulator. For example, animal experiments indicate that a SMF produced by a small magnet implanted near the carotid sinus (180 mT) for 6 weeks adds to the expected increase in plasma NO that would be produced by a calcium channel blocker(Okano and Ohkubo 2006). Although the SMF resulted in a slightly reduced blood pressure in comparison to sham treated rats, the BP reduction was greatest with the combined SMF and Ca^{++} blocker. Since BP reductions were experienced without changes in skin blood flow the BP reduction in this case may not be due to peripheral vasodilation. An absence of a skin blood flow effect despite a BP reduction was also seen when SHR were housed in cages for 12 weeks while exposed to a 5 mT SMF (Okano, Masuda et al. 2005). Contrastingly it has been argued that, at least in rabbits, there is an increase in peripheral vessel sensitivity to NO associated with SMF actions on carotid baroreceptors (Gmitrov 2013). Concentric magnets appear to have no significant effet on forearm blood flow in young adults (Martel, Andrews et al. 2002).

3.5 Human Skin Circulation as a Target

Despite the ability to non-invasively assess skin blood flow with the potential to systematically expose this tissue to various field intensity doses, human skin circulation as a target has been systematically studied by only a few investigators using SMF (Mayrovitz, Groseclose et al. 2001, Mayrovitz, Groseclose et al. 2002, Mayrovitz and Groseclose 2005, Mayrovitz, Groseclose et al. 2005, Li, Tam et al. 2007, Yan, Shen et al. 2011) and pulsed magnetic fields (Ueno, Lovsund et al. 1986, Mayrovitz and Larsen 1992, Mayrovitz and Larsen 1995, Mayrovitz, Sims et al. 2002, Wenzel, Reissenweber et al. 2005, McNamee, Corbacio et al. 2011, Kwan, Wong et al. 2015). The experimental data and concepts that has emerged from these and related investigations is worthy of

further in depth characterization in that it may serve to well illustrate and amplify various aspects not previously reported to a similar level of detail. In the following it is convenient to present these categorized by static and pulsed magnetic fields separately.

3.5.1 Static Magnetic Fields and Human Skin Circulation

A possible skin blood flow effect due to a SMF of a ceramic magnet placed on forearm skin was assessed in a group of young persons as depicted in **figure 2A**. The magnet, which had a surface B-field intensity of 95 mT, was in place for 35 minutes and was compared to the effects of a sham similarly placed (Mayrovitz, Groseclose et al. 2002). As shown in **figure 2B** the net result of this evaluation indicated no significant difference between LDF values associated with exposure to sham or to magnet. Another method to assess skin blood flow changes is by using laser Doppler imaging (LDI). In this method instead of placing a probe directly on skin, a laser beam scans skin areas of interest to determine LDI blood flow (Mayrovitz and Carta 1996, Mayrovitz and Leedham 2001). This method was used to assess possible SMF blood flow changes when one index finger was exposed to a magnet with B-field properties as shown in **figures 3** while the other index finger was exposed to a sham and various LDI scans run (Mayrovitz, Groseclose et al. 2001) as illustrated in **figure 4A-4C**. Over an exposure interval of 35 minutes there could be demonstrated no significant difference when exposed to sham or magnet with a surface B-field intensity of about 50 mT and a field gradient within the depth at which flow was measured dB/dz of about 4 mT/mm (**figure 4D**).

An extension of this work was to test the effect of an increased field intensity dose using neodymium magnets with surface B-fields near 0.4 T (Mayrovitz and Groseclose 2005). For this purpose finger dorsum LDF flow was measured simultaneously in finger 2 (F2) and finger 4 (F4) of the non-dominant hand while F2 rested on a magnet and F4 rested on a sham as shown in **figure 5A**. Exposure was for 30 minutes with an average finger thickness at the LDF flow site being 12 ± 1 mm (mean ±SD) which resulted in an average B-field dose at the F2 measurement site of 0.88 ± 0.05 T. Evaluations made in a group of 12 young healthy adults indicated a statistically significant decrease in LDF flow in the SMF exposed finger as compared to the sham exposed finger with a pattern as shown in **figure 5B**. The overall percentage reduction in LDF flow at F2 compared to pre-magnet exposure was near 18% as assessed after 30 minutes of exposure. Parenthetically an LDF flow reduction occurred independent of the pole (north or south) facing the skin. This appears to be the first reported finding of a SMF-related reduction in average skin blood flow.

It may be reasoned that if an SMF can affect average flow then it might also have an impact on vascular responses to perturbations. This aspect was assessed in several ways by evaluating the SMF affect on the LDF flow responses to inspiratory gasps (IG) and to vascular occlusions. The IG vascular

response (IGVR) relates to the transient reduction in skin blood flow that accompanies a rapid inspiration (inspiratory gasp). It is believed that this response is triggered by afferent sympathetic impulses to skin arterioles during rapid thorax and lung enlargement (Mayrovitz and Groseclose 2002). The concept to be tested using this perturbation is that a SMF alters the vasoconstriction response either by interfering with the afferent nerve traffic to the arteriole or by some how inhibiting the vascular smooth muscle's response.

This was tested using an experimental setup as shown in **figure 6** in which LDF flow was measured simultaneously on the 3rd finger dorsum of one hand which was exposed to a magnet while the other hand's 3rd finger was exposed to a sham magnet. After an interval of 20 minutes exposure of both fingers to sham, a series of IGs were performed. Thereafter one finger was exposed to the SMF of a magnet and the other to the sham for an additional 20 minutes and a series of IGs were again done. An example of the typical response for a single IG is shown in **figure 7**. The characteristic feature of the LDF flow response is a substantial reduction in flow in both fingers with a return to pre-IG levels. The response is quantified by calculating the percentage reduction in flow and this is termed the inspiratory gasp vascular response (IGVR). The net effect of this procedure on a group of 24 subjects (Mayrovitz, Groseclose et al. 2005) demonstrated essentially no difference between IGVR whether exposed to sham or magnet as illustrated in **figure 8**. In retrospect the inability to demonstrate a significant impact of the SMF on IGVR may in part have been due to the intensity of B-field at the finger dorsum LDF measuring site which for the previously described study averaged 31.5 mT. This author believes that the use of now available higher intensity magnets to clarify this issue would be a worthwhile future endeavor.

In contrast to a possible inhibition of a vasoconstriction response of IG, the question as to affects of a SMF on transient vasodilatory responses subsequent to an ischemic event was investigated using the experimental setup also as shown in **figure 6** but now simultaneously inflating both finger cuffs to produce an 5 minute full occlusion as illustrated with the response shown in **figure 9**. Ordinarily when tissue experiences an interval of blood flow and oxygen deprivation, which in this case was for 5 minutes, there is a subsequent immediate transient hyperemia that depends on the vasodilatory state of the tissue when the occlusion is released. The results of a series of assessments, of which **figure 9** is typical, suggest no significant effect on this hyperemia response as a result of 24 minutes of SMF exposure to the B-field intensity used.

However, experiments in which effects of a SMF on spectral content of LDF blood flow may indicate subtle changes in properties without measurable alterations in average flow. Such was reported in some experimental animal studies (Li, Tam et al. 2007, Xu, Okano et al. 2013) and has

been further investigated in human skin as depicted in **figure 10.** This represents an experimental setup to assess LDF spectral content changes when the middle part of the 3rd finger of a hand rests first on a sham magnet for 15 minutes and then on a 0.4 T surface field magnet for 30 minutes. Such measurements suggest a change in spectral content with an indication of a trend for decreasing mean LDF flow associated with the magnet as illustrated in **figure 11**. Such SMF-dependent changes in the spectral content of skin blood flow represent an interesting parameter for further study but at this time the presence of a significant SMF effect remains an open question.

One aspect to be considered in such investigations is the possibility of spectral components linked to specific physiological processes. For example applications of spectral and wavelet analyses suggest that there are at least five bands that present when recording skin blood flow with LDF (Kvernmo, Stefanovska et al. 1998, Kvandal, Landsverk et al. 2006). These bands include those due to endothelial cell activity (0.0095 – 0.02 Hz), neurogenic activity (0.02-0.06 Hz), myogenic activity (0.06-0.15 Hz) and respiratory activity (0.15-0.4Hz). There is also usually a band related to the heart rate (0.6-1.6 Hz). In an initial attempt to probe the possible impact of magnetic fields on the skin blood flow spectrum LDF was measured on the dorsum of fingers 3 and 4 while exposed to an SMF of 0.12 T produced by magnets secured to a turn table as shown in **figure 12**. LDF flow was generally recorded for 8 continuous minutes before altering the number of magnets placed on the rotating turn table with each 8 minute sample subjected to spectral analysis. The concept under test with such an arrangement would be that if there were any impact of the magnet on LDF flow then there should be a spectral component associated with that of the impulse rate. Some sample spectra are shown in **figure 13,** which appears to indicate a shift in the spectrum during magnet exposure at a rotation frequency of 0.333 Hz but the full interpretation must await further data accumulation and analysis. However, the spectral analysis approach to the question appears to a useful tool and is currently being investigated.

3.5.2 Pulsed Magnetic Fields and Human Skin Circulation

One major difference to consider when discussing pulsed electromagnetic fields (PEMF) is the fact that the time changing component gives rise to induced currents within target tissues and their surround. A possible coupling of various forms of PEMF to circulation related parameters and processes has been the subject of considerable discussion (McKay, Prato et al. 2007, Pilla 2015) with various applications (Markov 2007) and some evidence of a PEMF induced vascular smooth muscle relaxation possibly involving a calcium dependent-nitric oxide process (Pilla 2012) although no such process has been specifically shown to effect skin circulation. However, there is evidence that skin circulation can be altered by exposure to PEMF even if the involved mechanisms are unclear. Early

workers used a form of PEMF in which a base band time varying signal modulated a radio frequency carrier typically at 27.12 – 27.33 MHz which are diathermy frequencies (Guy, Lehmann et al. 1974). Application of such signals when at a sufficiently low intensity was by some termed “athermic” (Liebesny 1938) to suggest that effects observed were not attributable to tissue heating but this may not be confirmed in all cases (Silverman 1964, Silverman and Pendleton 1968) even if short duty cycles are used. Thus some early work using PEMF may in part be related to mild heating. None-the-less, there are reports of increased lower extremity blood perfusion indices in persons free of vascular disease (Erdman 1960) and in persons with peripheral arterial disease (Hedenius, Odeblad et al. 1966, Valtonen, Lilius et al. 1973). Further, direct measurements of skin blood perfusion in arms of healthy persons (Mayrovitz and Larsen 1992) and in persons with lymphedematous arms (Mayrovitz, Sims et al. 2002) and with lower extremity ulcers (Mayrovitz and Larsen 1995) indicate a PEMF related increase in blood flow.

An important contribution to clarifying the question of heat vs. EMF effects on vascular targets was addressed, not in human skin, but by exposing blood vessels in the web of the frog to PEMF of 0.1, 1 and 10 MHz at burst rates of 10KHz at 50% duty cycles for up to 60 minutes at magnetic and electric field strengths at the target calculated to not raise tissue temperature by more than $0.036^{o}C$ (Miura and Okada 1991). When topical NE was used to pre-constrict microscopically observed arterioles to about half their resting diameter, exposure to PEMF at each frequency caused diameters to increase after 10-20 minutes of exposure with a peak vasodilation at about 60 minutes. The 10 MHz excitation produced the largest vasodilation (to about 68% of pre-constriction) but was not significantly different than that caused by 1 MHz excitation. The amount of vasodilation was dose-dependent for pre-constricted arterioles and for non-pretreated arterioles. Percentage increases in arteriole diameters for non-pretreated arterioles were on average 12% and 26 % for target magnetic field intensities reported as 7.3 mG and 30.1 mG and electric field intensities of 2.19 v/cm and 9.00 v/cm with pre-constricted arterioles dilating more for the same dose.

Based on manipulations of the suffusing fluid content the authors concluded that the PEMF vasodilation depended on Ca^{++} efflux from VSM and/or influx into VSM sarcoplasmic reticulum both well known processes that alter VSM tone as discussed in earlier sections of this chapter. Based on prior work (Miura and Okada 1988, Okada and Miura 1990) they hypothesized that the PEMF facilitates activation of the Ca^{++}-ATPase calcium pump thereby modulating VSM tone. Subsequent work provided evidence of a strong involvement of nitric oxide via a PEMF-induced cyclic GMP activation when cerebella tissue was exposed to a similar 10 MHz, pulsed field (Miura, Takayama et al. 1993). Another microscopic observational animal study using PEMF for which heat was reported as

a non-issue was done on rat cremaster circulation (Smith, Wong-Gibbons et al. 2004). Exposing the cremaster to a pair of Helmholtz coils with approximate 3.7 KHz pulses (pulse width of 5.9 ms and duty cycle of 8.8%) resulted in arteriole vasodilation of 9% after only 2 minutes of exposure although the field intensity at the target was not specified. These PEMF-related diameter changes can have substantial flow effects but it is unclear if they are due to induced currents, electric field changes that affect membrane potentials or to other processes since it is well established that various forms of electrical stimulation result in altered skin blood flow with increases reported in skin of forearm (McDowell, McElduff et al. 1999, Cramp, McCullough et al. 2002) and leg (Noble, Henderson et al. 2000). Further study is clearly warranted.

Conclusion

Whether or not all or any of the mechanisms are fully understood it would appear that based on the cited materials and approaches spanning many years and the current analysis of these findings, both SMF and PEMF are modalities that have variable impacts on vascular blood flow and related hemodynamics. There is thus good reason to further explore the way in which differences in doses and field arrangements in the case of SMF and differences in parameter values in the case of PEMF affect vascular components and blood flow in humans.

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Figure 1A return1 return2 return3

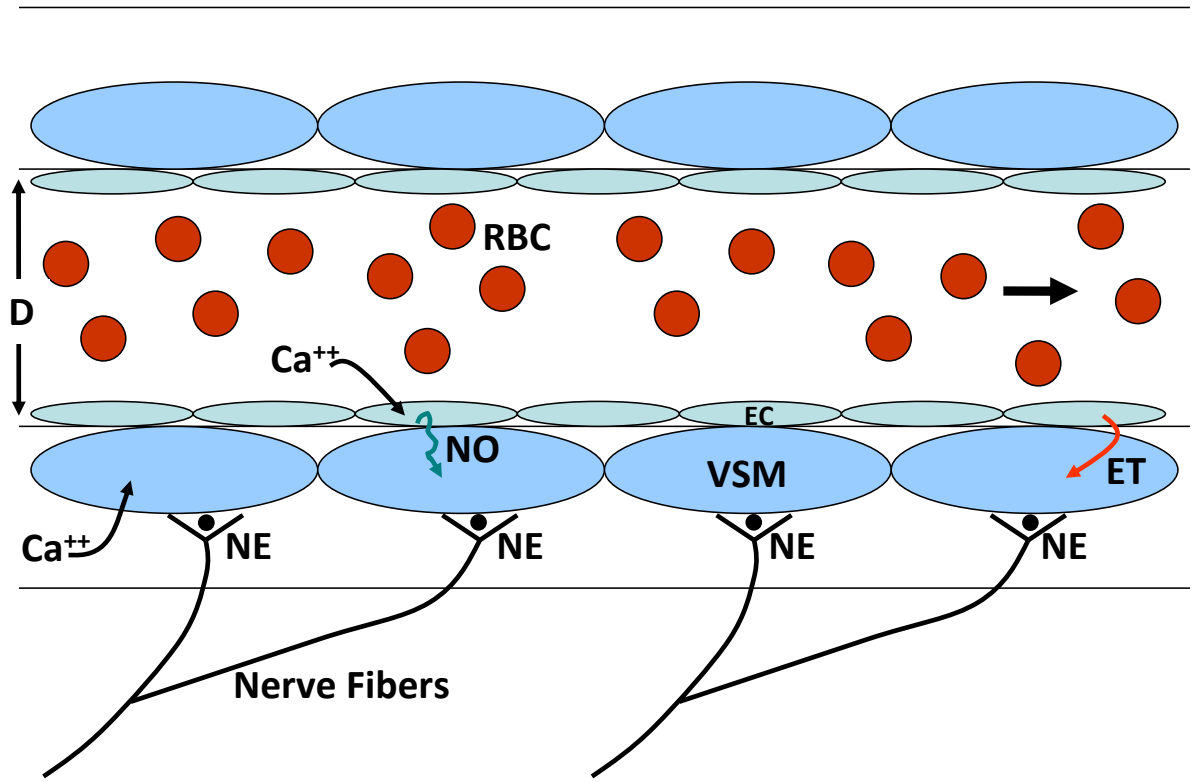

Figure 1A. Simplified Schema of Potential Vascular Related Targets

RBC = red blood cells, D=vessel diameter, VSM = vascular smooth muscle, EC = endothelial cells, NO = nitric oxide (a potent vasodilator), ET = endothelium (a potent vasoconstrictor), NE = norepinephrine (released from many sympathetic nerves main peripheral vessel action is vasoconstriction).

Figure 1B return

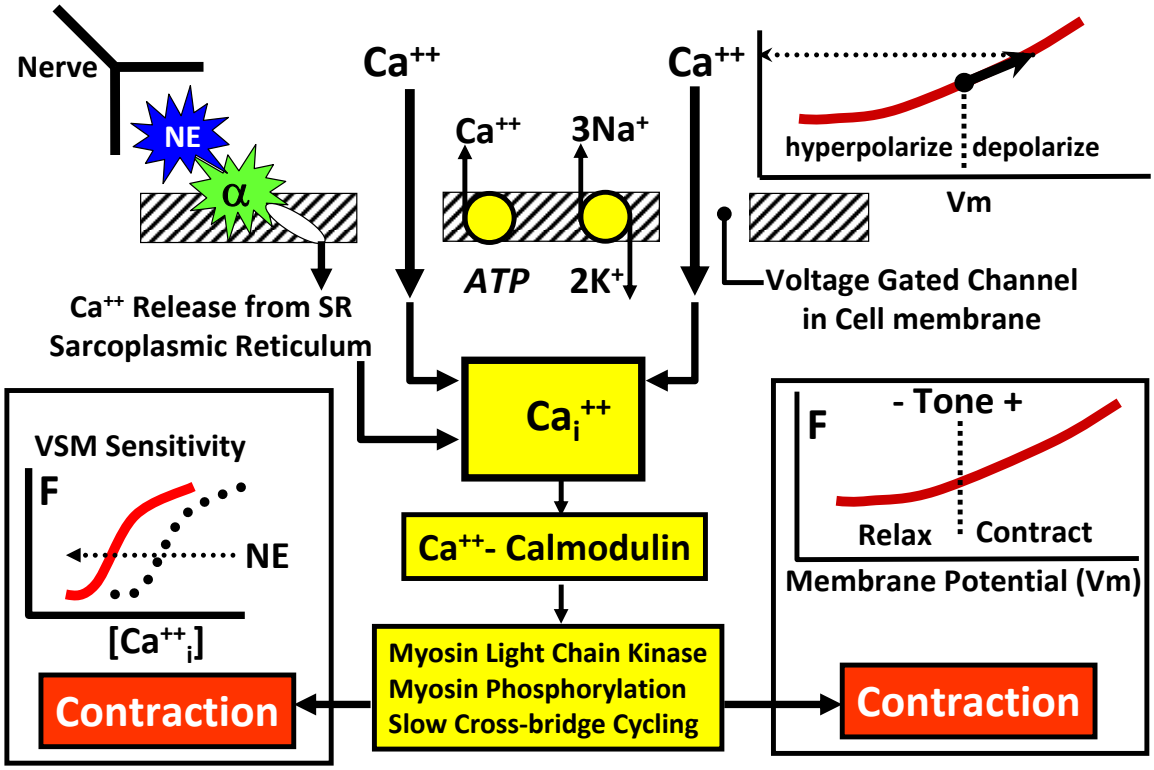

Figure 1B. Overview of Some Calcium Related Contraction Mechanisms as Potential Targets

VSM resting membrane potential (Vm) is mainly determined by high membrane K^+ permeability and outward K^+ current. Internal [K^+] is maintained by active $3Na^+$-$2K^+$-ATPase pump. More Ca^{++} enters through voltage gated channels if cell membrane depolarization occurs. Additional Ca++ entry is also triggered by ligand gated channels such as that caused by the release of NE from nerve terminals acting on α-receptors. Ca^{++} release from SR stores makes more Ca^{++} available and promotes vasoconstriction whereas Ca^{++} removal by the SR and membrane pumps reduces Ca^{++} availability promoting less vasoconstriction (vasodilation). Sensitivity of VSM contractile machinery to Ca++ concentration [Ca^{++}] increases with increased [NE] enhancing VSM contraction Force (F).

Figure 1C return

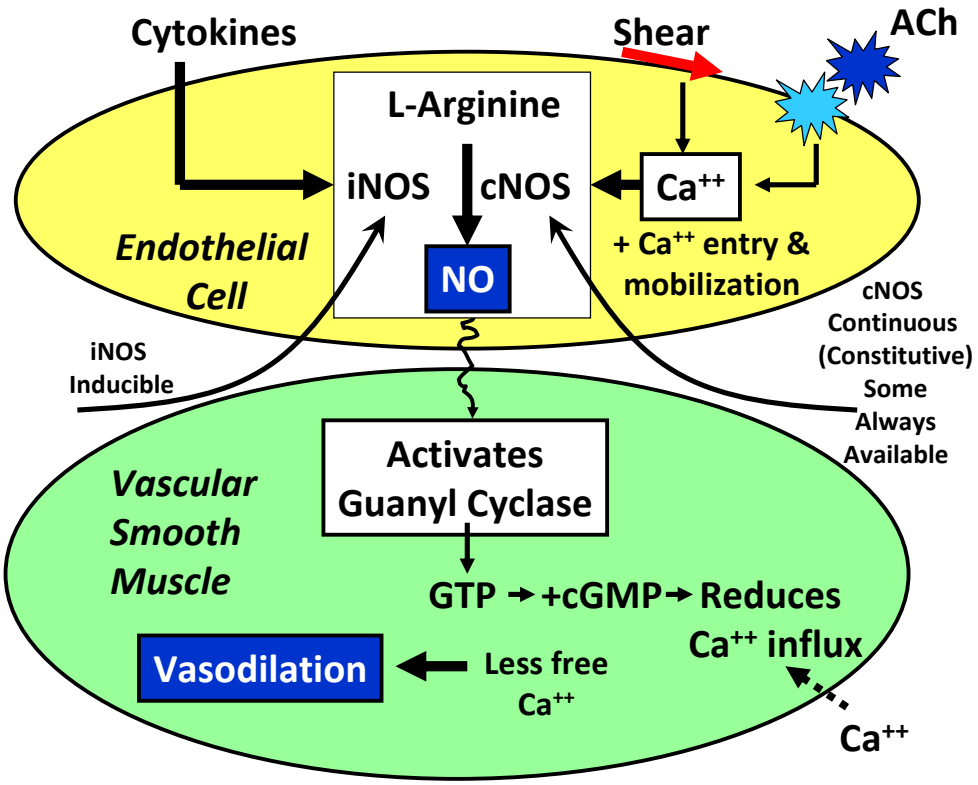

Figure 1C. Overview of Some Endothelial Cell Related Mechanisms as Potential Targets

If Ca^{++} influx is increased in endothelial cells, biochemical processes are triggered that include more release of nitric oxide (NO) that diffuses to VSM causing these cells to be less contracted (more relaxed) that generally results in an increase in the vessel's diameter (vasodilation). This effect depends on EC synthesis and release of NO induced by chemical and physical stimuli including acetylcholone (Ach) andwall shear stress. NO is synthesized from L-arginine in the presence of constitutive nitric oxide synthase (cNOS) that is Ca^{++} dependent and inducible nitric oxide synthase (iNOS) that depends cytokines and other substances. In VSM NO activates cytoplasmic guanosine tripohsphate (GTP) that then increases cyclic guanosine monophosphate [cGMP], causing a decrease in Ca^{++} entry and a decrease in cytosolic free Ca^{++} in VSM resulting in relaxation.

Figure 2 return

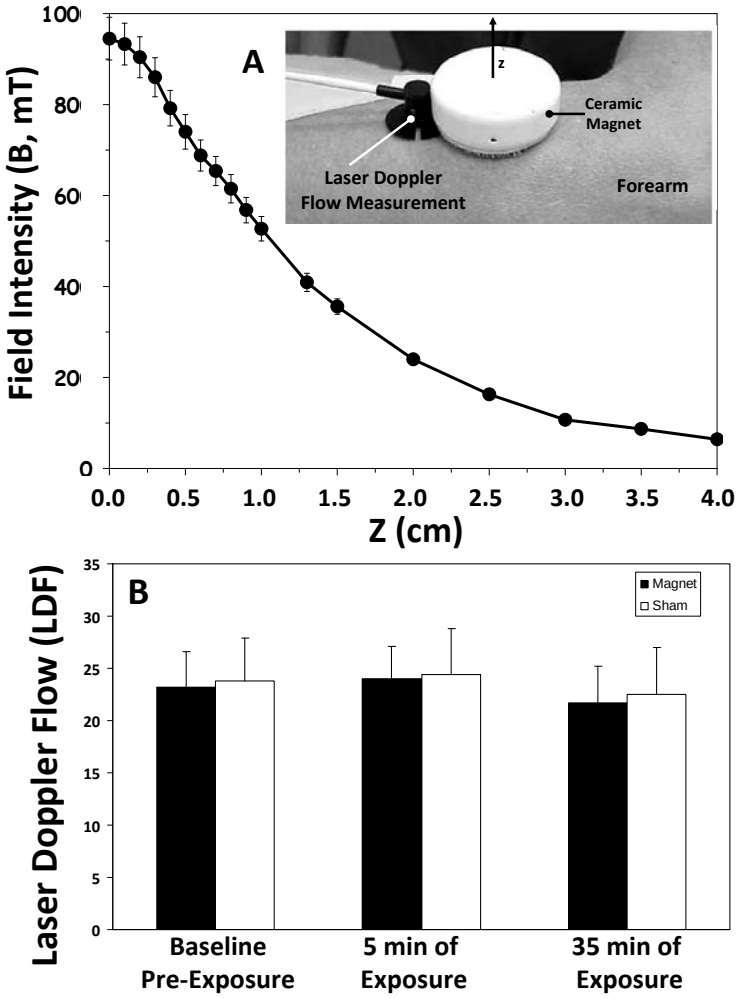

Figure 2. Measurement of forearm skin blood flow with and without a SMF.

(A) A ceramic magnet with a B-field intensity as shown in the figure or a sham magnet was placed on the forearm of a group of 12 healthy subjects for 35 minutes and the laser Doppler flow (LDF) within 5 mm distal to the magnet edge monitored continuously. Results (B) show mean and sem for LDF that demonstrated no difference between sham and magnet exposed.

Figure 3 return

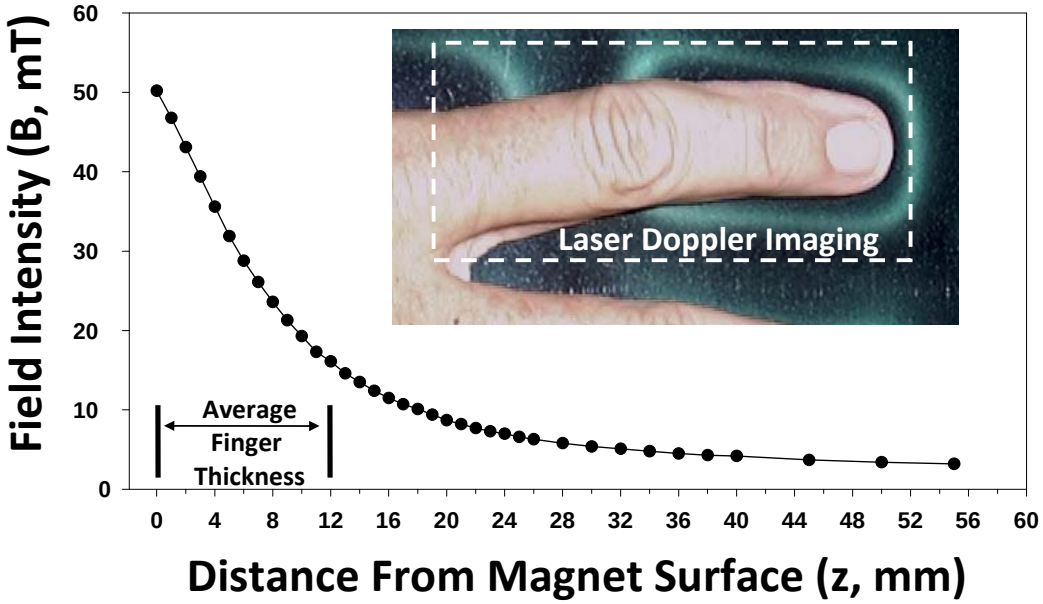

Figure 3. Laser Doppler Imaging (LDI) Setup of Finger Dorsum Exposed to Magnetic Pad

Index finger is shown lying on a rectangular magnet with the B-Field intensity as shown in the figure. The finger dorsum blood flow is measured to a depth of about 1.5 – 2.0 mm via scanning the finger surface over a time span of about 4 minutes per scan. In practice one index finger lies on a magnet and the other on a sham that are both scanned simultaneously as shown in figure 3. Over the blood flow measurement depth the B field gradient is approximately 4 mT/mm.

Figure 4 return

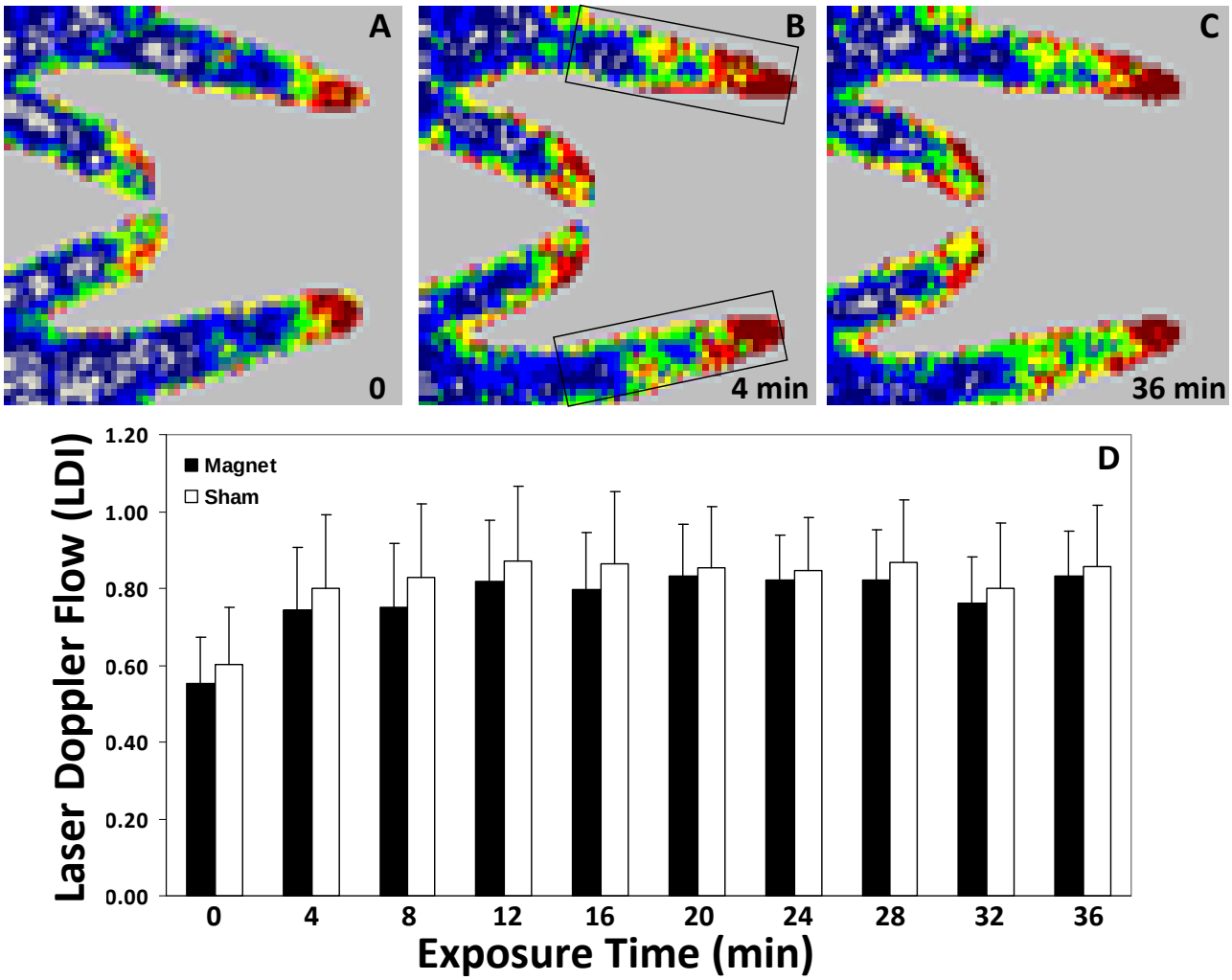

Figure 4. LDI Scan Results for Magnet and Sham Exposed Index Fingers

A, B and C show an example scan for baseline (A) after 4 minutes of exposure (B) and after 36 minutes of exposure. In this example the upper finger is exposed to the magnet. In part D the summarized results (mean + sem) are shown for the evaluated group indicating no significant difference in LDI flow between sham or magnet exposed.

Figure 5 return

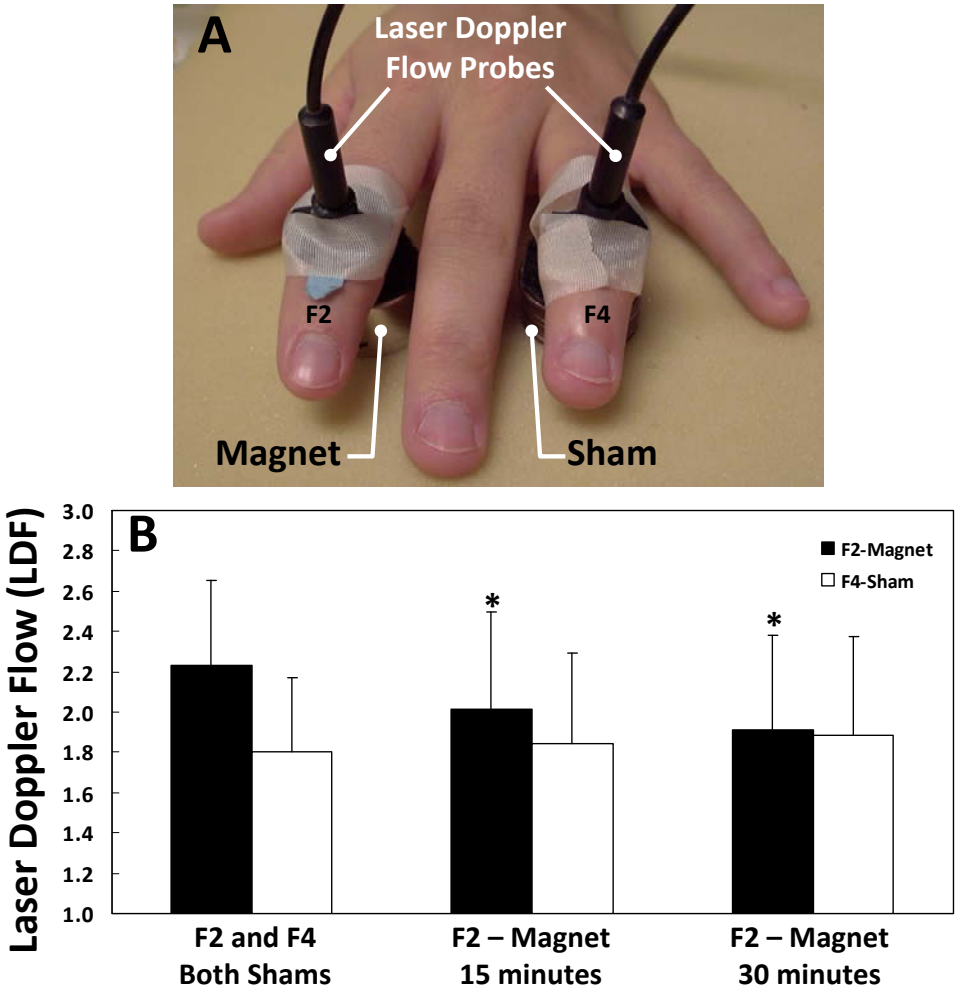

Figure 5. Simultaneous laser Doppler Flow Measurements with Neodymium Magnet Exposure

(A) Setup for measuring finger dorsum LDF flow simultaneously in fingers F2 and F4 of the non-dominant hand. F2 was supported by magnet with surface field of 0.4 T. Average measured B-field at F2 dorsum target was 0.88 T. (B) Results (mean + sem) for 15 and 30 minute SMF exposure in a group of 12 young adults indicate a statistically significant decrease in LDF flow as compared to the sham exposed finger.

Figure 6 return1 return2

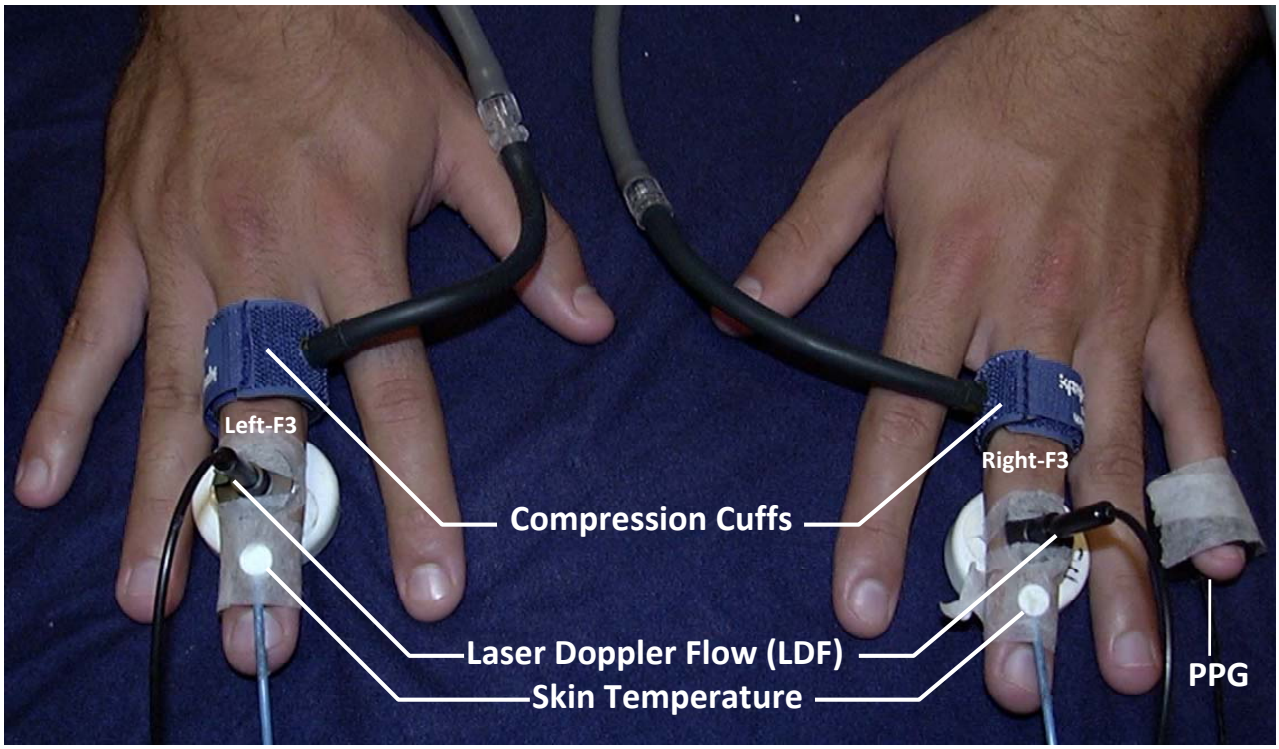

Figure 6. Experimental Setup to Evaluate SMF Affects on Vascular Responses

Laser Doppler flow (LDF) is measured simultaneously on the 3rd finger dorsum while fingers rest on either a magnet or a sham. The pulse is continuously monitored with the PPG and finger skin temperatures are monitored with the thermocouple placed just distal to the flow probes. The compression cuffs around the base of the fingers are used to produce a full blood flow occlusion.

Figure 7 return

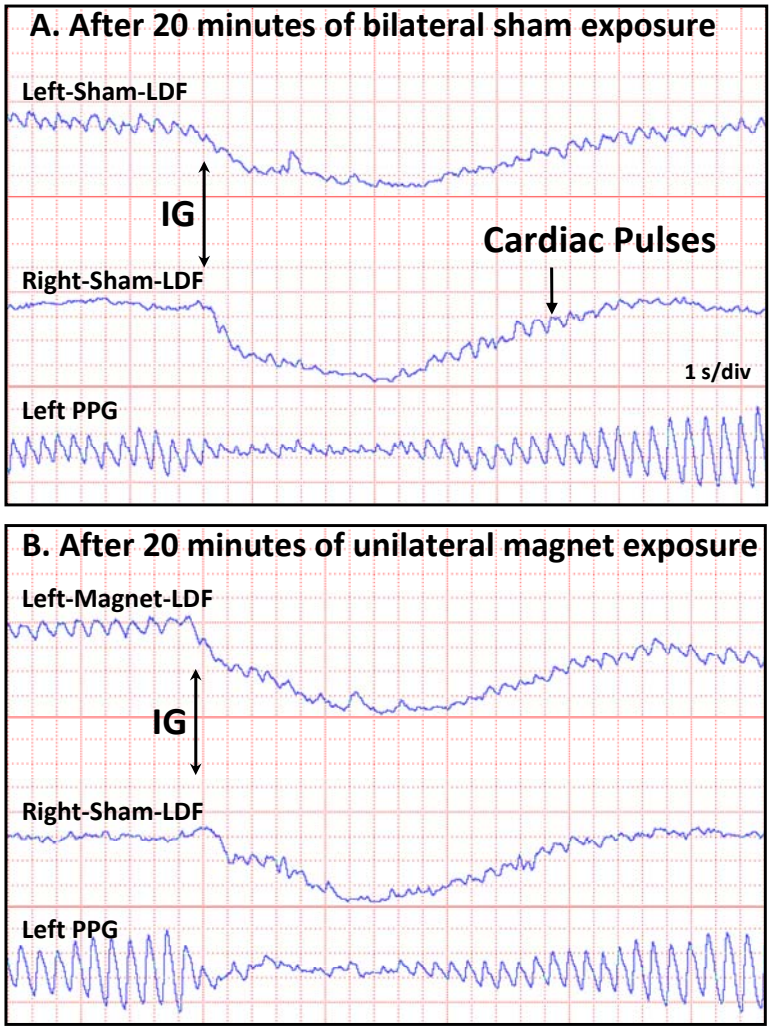

Figure 7. Example Blood Flow responses to Inspiratory Gasps

(A) Bilateral IG responses after both middle fingers exposed to shams for 20 minutes

(B) Bilateral IG responses after left finger exposed to magnet and right exposed to sham for 20 minutes. There is essentially no difference between sham and magnet exposed in this subject.

Figure 8 return

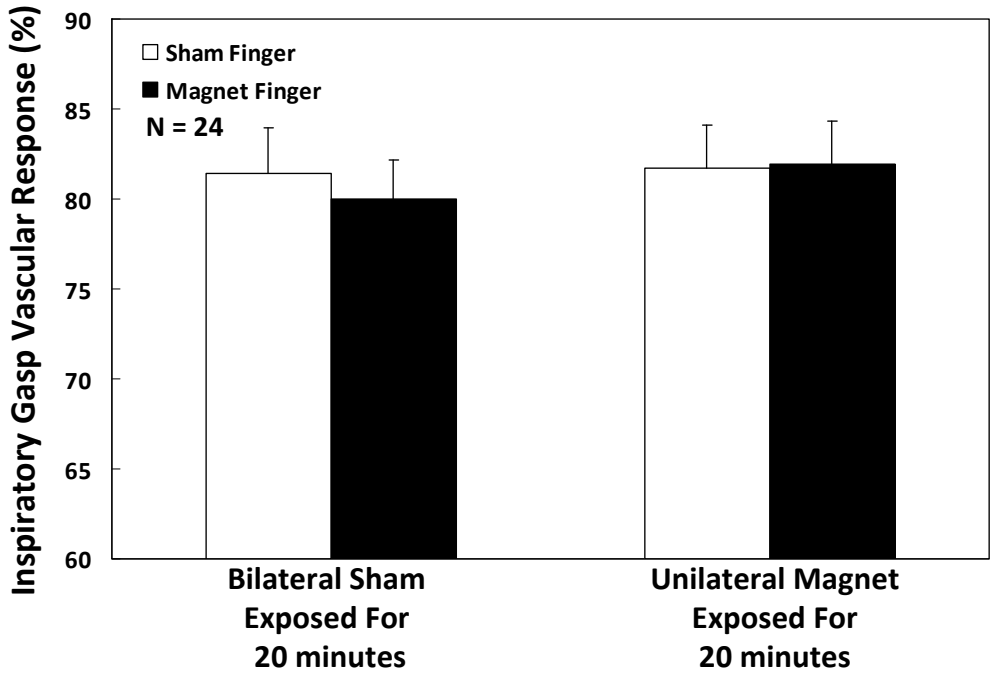

Figure 8. Composite Results for Inspiratory Gasp Vascular Responses (IGVR)

The IGVR is the percentage reduction of the mean LDF flow caused by a rapid inspiration. The figure shows the overall mean + sem IGVR for 24 healthy subjects first exposed bilaterally to sham and then unilaterally to a magnet with the field properties similar to that shown in figure 1A. There was no difference in vascular responses whether exposed to sham or to magnet.

Figure 9 return

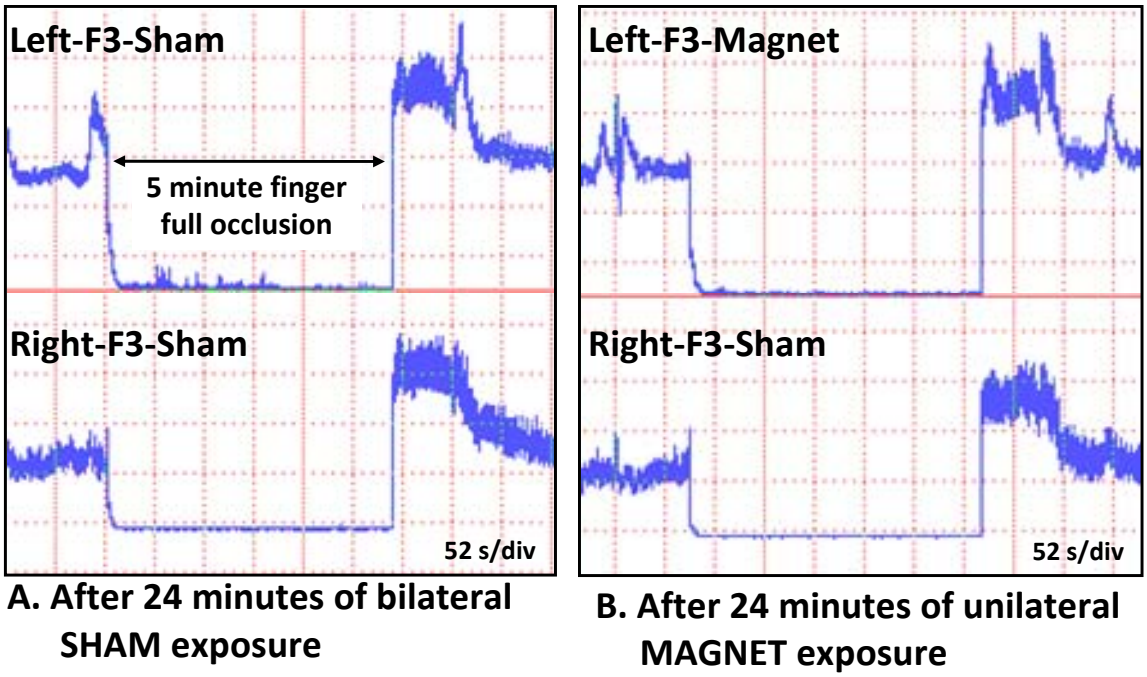

Figure 9. Example Blood Flow Responses to Full Vascular Occlusion

(A) Inflation of the finger cuff for 5 minutes on the 3rd finger of both hands after both fingers exposed to shams as shown in figure 5. (B) Cuff inflation and release after left 3rd finger exposed to 24 minutes of a SMF produced by the magnet shown in figure 5. The hyperemic response to the prior flow stoppage was essentially the left finger was exposed to sham or magnet. This basic response for this subject held for a group of 20 subjects similarly evaluated.

Figure 10 return

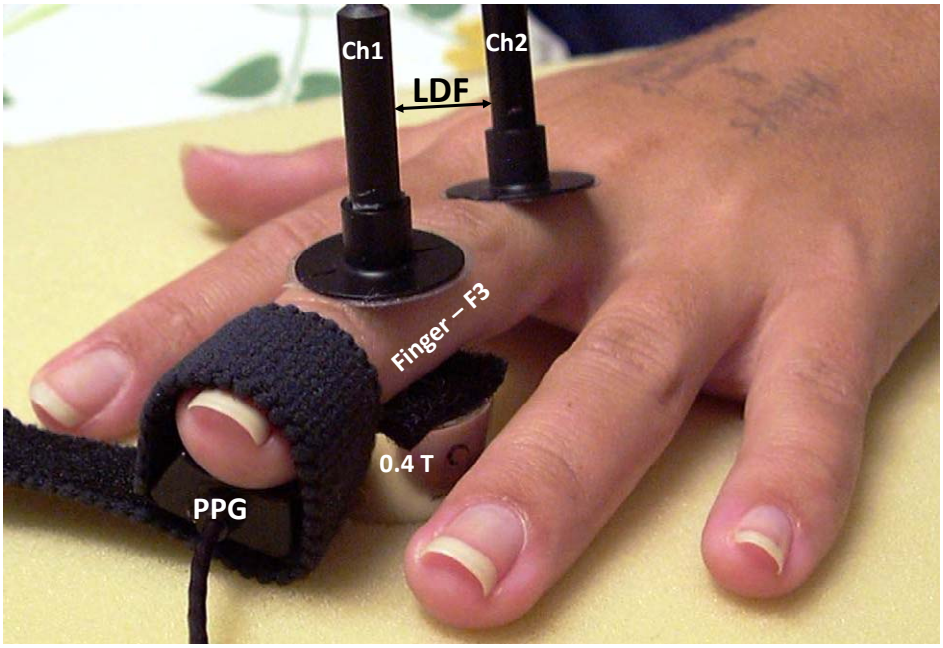

Figure 10. Experimental setup to assess LDF spectral content changes

Middle part of the 3rd finger of non-dominant hand rests on either a 0.4 T magnet or a sham while laser Doppler blood flow is continuously recorded at the magnet exposed finger dorsum (ch1) and on the same finger proximal to it (ch2). Resultant LDF signal is subjected to spectral analysis as illustrated in figure 11.

Figure 11 return

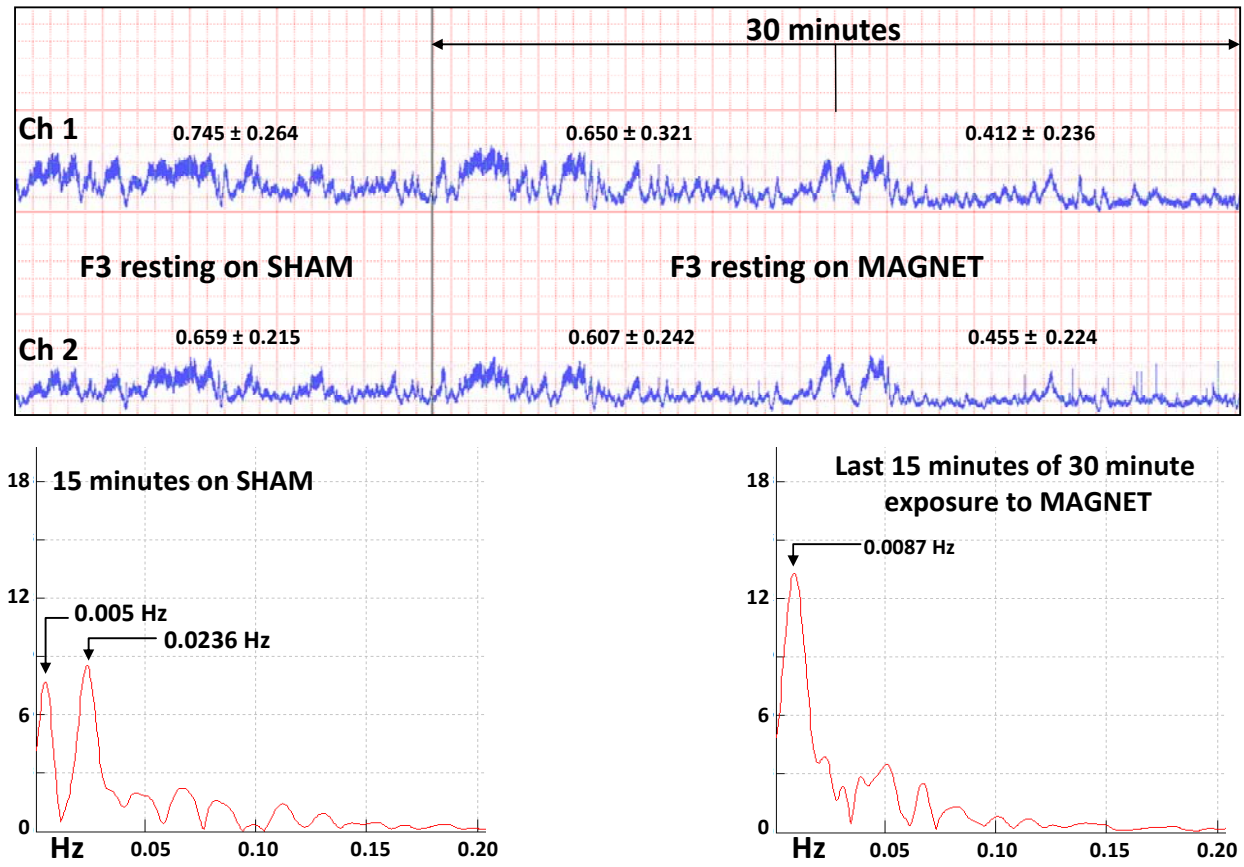

Figure 11. Example LDF flow recording and associated frequency spectrum.

F3 initially rests on sham for 15 minutes and then the sham is replaced by a 0.4 T magnet that in this example resulted in a measured 0.09 T at the dorsum LDF measurement site. The similarity in Ch 1 and Ch 2 LDF temporal patterns is evident. The substitution of the sham for the magnet seems to indicate a shift in the spectral content. Numbers in Ch 1 and Ch 2 indicate the LDF average during each of the consecutive 15 minute intervals (mean ± SD).

Figure 12 return

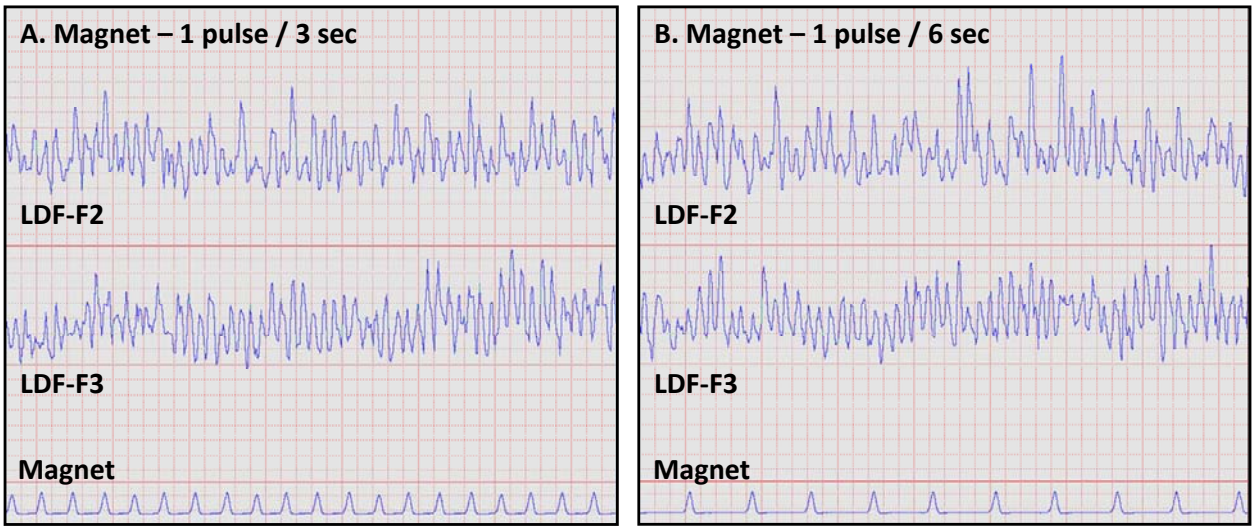

Figure 12. Setup and Example for Rotating Magnet Exposure

The subject's hand rests on the wood block while the turntable rotates exposing fingers 2 and 3 to the fields of the rotating magnets. Average field experienced at the finger dorsum was 0.12 T that was produced by each of the magnets. The rotation speed (Ω) was fixed and the rate of exposure was determined by the number of magnets that were placed on the turntable. Parts A and B are example recordings each of 60 seconds long showing LDF on F1 and F2 with 4 magnets placed (A) and two magnets placed (B).

Figure 13 return

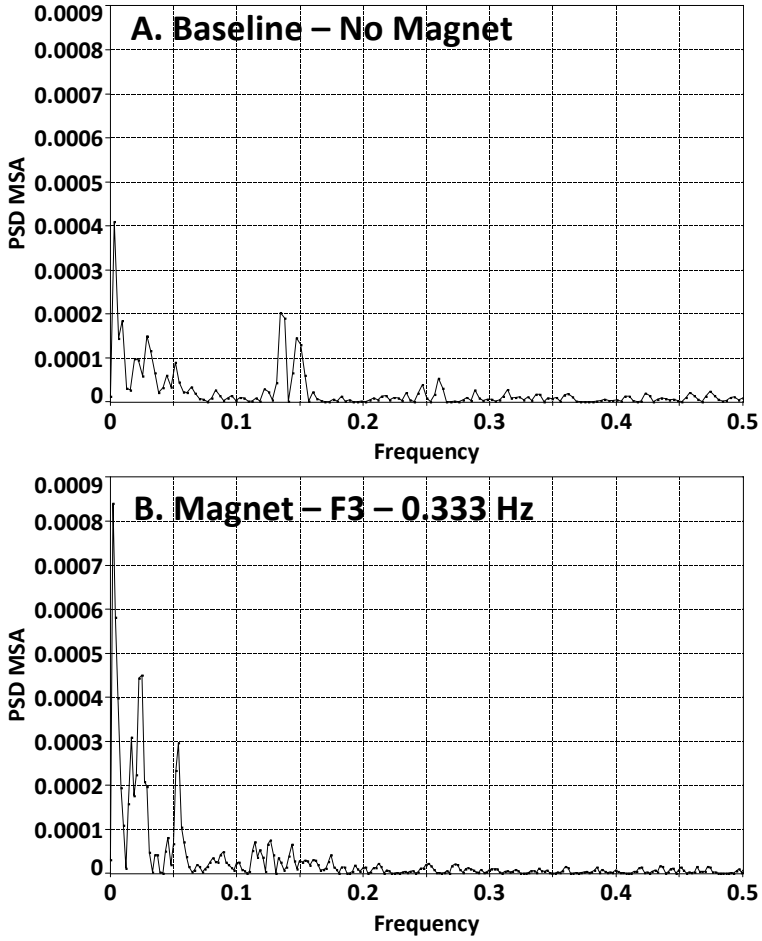

Figure 13. Power Spectral Distribution of LDF Without and With Rotating Magnets

(A) Power spectral density (PSD) of baseline LDF for finger F3. (B) PSD of 8 minute exposure to rotating field (1 impulse / 3 seconds = 0.333 Hz) as exemplified by the sample of figure 11A. The measured peak B-field measured at the finger dorsum was 0.125 T.