Laser Doppler Flowmetry in Manual Medicine Research

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Laser Doppler flowmetry (LDF) is commonly used in combination with reactivity tests to noninvasively evaluate skin sympathetic nerve activity and skin microvascular function. In manual medicine research, LDF has been used as a marker for global peripheral sympathetic nervous system function, but these results should be considered with caution because skin sympathetic nerve activity physiology is often overlooked. Another limitation of LDF in manual medicine research is the processing of LDF recordings. Two methods have been suggested: the time-domain analysis and the frequency-domain analysis. Standardization is required for data collection and processing in either domain to accurately interpret these changes in skin blood flow that occur after manual procedures. For physiologic studies using LDF, the authors recommend the use of noninvasive reactivity tests (positive controls) to evaluate the different mechanisms involved in overall skin blood flow changes and to compare the magnitude of these changes with those specifically elicited by manual procedures.

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Andrew Taylor Still, MD, DO, emphasized in his writings the crucial role of osteopathic manipulative treatment (OMT) in maintaining a normal flow of body fluids, especially blood flow, as a means to recover from disease and maintain health.[1] From its inception, the osteopathic medical profession has investigated the OMT-induced physiologic changes to add scientific evidence to positive clinical outcomes. In 1907, Burns[2] published the first article on peripheral sympathetic nervous system (PSNS) reflex changes in animal models and humans. In a literature review, Bolton and Budgell[3] investigated the initial physiologic changes observed after different types of spinal manipulation, mainly through the markers of PSNS function.

The most common noninvasive markers used to investigate changes in PSNS have been indexes of skin blood flow (SBF). Among these indexes, laser Doppler flowmetry (LDF) has been studied in manual medicine research.[4-11] Although it requires control of several parameters, LDF is easy to implement and has the potential to advance OMT research. This equipment has been used in pharmacologic studies to evaluate endothelial and neurovascular function in the skin microcirculation.[12] Laser Doppler flowmetry signals can be processed with time-domain or frequency-domain analyses, but no consensus has been reached on the best way to express these data.[13] Thus, the use of LDF presents several methodologic challenges that need to be overcome for an appropriate understanding of the recordings.[14] For example, studies have overlooked the specificities of skin sympathetic nerve activity (SSNA) that control SBF,[3] so correlating manual procedures with changes in PSNS func-

tion from the measurements of SBF recorded with LDF equipment may be inaccurate.

There have been inconsistencies in the literature regarding LDF use in manual medicine research. Therefore, the purpose of this narrative review is to present recent physiologic information on how SSNA controls SBF, how LDF signals have been processed with time-domain and frequency-domain analyses, and how these physiologic changes have been interpreted by manual medicine researchers. Laser Doppler flowmetry data may contribute to an evidence base for OMT and may aid osteopathic physicians in the clinical application of OMT.

Initial Neurophysiologic Changes Associated With Manual Procedures

Early osteopathic researchers considered SBF indexes as potential markers for PSNS function[15,16] and studied somatosomatic and somatosympathetic reflex changes associated with abnormal findings of musculoskeletal structures in the spine as detected by palpation.[17,18] Based on sudomotor and skin temperature studies,[17,18] their results supported the first theoretical models for initial physiologic changes associated with OMT. These models were mainly segmental in nature because OMT was supposed to elicit segmentally related spinal somatosympathetic reflexes in superficial and deep tissues and to influence specific visceral function at the corresponding spinal levels,[19] which was empirically described by early osteopathic physicians.[1] Researchers in manual medicine have proposed comprehensive literature reviews on the physiologic changes associated with spinal manipulation and mobilization (SMM).[20-22] For instance, SMM has been considered to have a neurophysiologic mechanism of action through the stimulation of the dorsal periaqueductal grey matter in the midbrain and its descending pathways, which excite and produce nonopioid analgesia by specifically suppressing the mechanical nociceptive stimuli and producing a general pattern of sympathoexcitation.[22,23]

Further, SMM is usually associated with initial sympathoexcitatory effects as recorded by changes in respiratory rate, heart rate, heart rate variability, blood pressure, pupillary light reflexes, inflammatory and immune responses, and by changes in several indexes of SBF. The most commonly used SBF indexes are skin conductance, skin temperature, pulse plethysmography, and LDF.[3,24] Compared with pulse plethysmography, cutaneous electrical conductance, and skin temperature, the use of LDF recordings in manual medicine is relatively new, and outcomes are inconsistent: SMM has been associated with an initial decrease or increase in SBF.[3] These differences could be because of the high variability of the LDF signal, the small sample sizes of the studies, variability in how the data were collected, and inconsistency among researchers in processing and interpreting the LDF signals. The construct validity of using skin conductance and skin temperature as measures of PSNS function has been discussed by Moulson and Watson.[25] The authors concluded that skin temperature seems to be a more sensitive and reliable measure of vasomotor function than PSNS and that skin conductance is prone to variability because it can be affected by psychologic and personality factors.[25] To our knowledge, no such critical analysis of the use of LDF equipment has been published.

Indexes of SBF and SSNA

Because of their noninvasive nature and because they were thought to describe similar changes in deeper tissues, indexes of SBF have been extensively used as markers of PSNS. The initial response to SMM was described as a peripheral vasoconstriction of the arterioles within the dermis and a decrease in SBF leading to a decrease in skin temperature and an increase in skin conductance.[26,27] However, the underlying mechanisms are not fully understood, and this hypothesis is now considered overly simplistic because SBF indexes cannot be substituted for direct measurements of PSNS function,[3]

and because the specificities of SSNA and their influence on SBF regulation have not been explicitly described in previous manual medicine publications, to our knowledge. The SSNA comprises 4 different nerve categories: vasoconstrictor, vasodilator, sudomotor, and piloarrector; muscle sympathetic activity comprises only vasoconstrictor nerves.[28] This physiologic difference might explain why muscle sympathetic nerve activity, as evaluated with invasive microneurographic techniques, is considered a better marker of PSNS activity than SSNA.[29] Activation of SSNA nerves depends on the thermal environment and the level of physical and emotional activity.[28] Further, the sympathetic vasoconstrictor system is tonically active in thermoneutral environments, but its influence is not substantial because SBF is relatively low at rest. The sympathetic vasodilator system, which is not tonically active, is activated only during increases in internal temperature, such as during exercise or environmental heat exposure.[28] The SSNA nerves exert their vasoconstrictor and vasodilator influences over vascular smooth muscles and the arteriovenous shunt to deviate blood flow to regulate body temperature; the shunt is closed to radiate heat in hot environments and open to conserve heat in cold environments (*Figure 1*).

LDF: General Methods and Applications

Laser Doppler flowmetry is a noninvasive technique for monitoring and evaluating red blood cell velocity and concentration, providing an index of perfusion often referred to as *flux*. The infrared light from a low-power laser is directed via an optical fiber onto the tissue to be studied, and the light scattered back from the tissue is collected by 1 or more other optical fibers and analyzed.[30] When applied to the skin, the infrared light penetrates the tissue 1.0 to 1.5 mm to measure cutaneous microvascular blood perfusion in the dermis (*Figure 1*).[4] Coupled with a reactivity test, which is a mechanical, thermal, electrical, or pharmacologic stressor eliciting a known and reproducible physiologic response, LDF has been used to investigate peripheral microvascular disorders commonly seen in diabetes mellitus, atherosclerosis, kidney dysfunction, hypertension, and heart disease.[12] Among reactivity tests, the postocclusive reactive hyperemia test and the local thermal hyperemia test have a well-established validity.[12] Specific noninvasive evaluations of the influence of SSNA on SBF (vasoconstrictor reflex) include respiratory tests such as the inspiratory gasp test, thermal tests such as the cold pressor test, mental tests such as the Stroop test, and noxious stimuli recorded with LDF equipment.[31]

When a time-domain analysis is used, the LDF data are expressed as arbitrary perfusion units (APU), and values are averaged over a specific period and then expressed as a percent change from baseline. The LDF signal can also be filtered and transformed into a frequency domain using a power spectral analysis (*Figure 2*). The LDF spectra can be divided into 5 components: heart activity (2.0-0.6 Hz), respiratory activity (0.6-0.15 Hz), vascular myogenic activity (0.15-0.06 Hz), neurogenic (sympathetic) activity (0.06-0.02 Hz), and endothelial activity (<0.002 Hz).[32] These 5 different components are supposed to represent the specific factors that affect overall changes in SBF (*Figure* 3). The frequency ranges and physiologic origins of these components are almost identical to those of heart rate variability, except that the neurogenic and endothelial components are usually not separated in the latter.[32] However, there is controversy about which method—time domain or frequency domain—is more appropriate to describe changes in LDF signals.[13]

LDF Recordings in Manual Medicine

In the 8 published papers that evaluated changes in LDF recordings after manual procedures, investigators recorded SBF changes at different anatomic locations: the upper limb, the lower limb, or the head. In the upper limb, investigators used 2 probes at 2 locations: 1 over

the thumb and 1 over the lateral epicondyle[11] or 2 probes over the same forearm.[5] In the lower limb, investigators used 2 probes: 1 over each dorsum of the foot over the dermatome of the fifth lumbar vertebra.[4] In the head, investigators used 1 probe at 1 location: over 1 earlobe,[6] over the left earlobe,[8-10] or over the midline of the forehead.[7] In these studies, the authors did not discuss the possible influence of constitutional factors, such as age, sex, skin pigmentation, skin type, or smoking habits, that may influence LDF recordings,[33] thus making it difficult to extrapolate results to the general population.

The positioning of the LDF probes can also influence SBF variability.[14] The epidermis is the most superficial layer of the skin and contains no blood vessels. Its depth varies from 0.05-mm thick on the eyelids to 1.5-mm thick on the palms of the hands and the soles of the feet. Under the epidermis, the depth of the dermis varies from 0.3 mm on the eyelid to 3.0 mm on the back (*Figure 1*). Thus, the LDF beam may not be able to reach the dermis in certain areas of the body.[34] The studies we reviewed did not address this issue as it pertained to the most common sites used for LDF recordings.[12,14] The best data are usually obtained when the LDF probe is taped over skin that has the highest density of arteriovenous anastomosis within the 1.0- to 1.5-mm depth range of the laser beam. When SBF was assessed with a single-point LDF probe placed on the finger pad or fingertip skin, the reproducibility was higher compared with forearm placement because of the higher density of arteriovenous anastomoses in the finger pads.[14,35]

Paungmali et al[9] and Vicenzino et al[11] discussed concurrent opposite changes in SBF in the hand and elbow caused by possible different control mechanisms in glabrous or hairy skin. Mechanisms influencing SBF are currently better documented,[14] but data showing possible differences in SBF after SMM between glabrous and hairy skin are lacking. Roustit and Cracowski[12] recommend the use of a vacuum cushion to keep the hand and forearm as still as possible to reduce motion artifacts, but this procedure might be challenging for

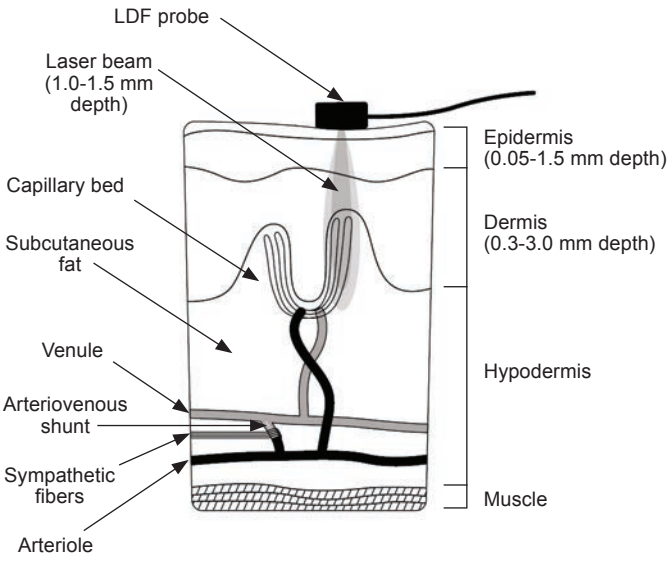

Figure 1.
Microvascular perfusion as measured with laser Doppler flowmetry (LDF). The laser beam penetrates the dermis to measure the number of moving red blood cells and their mean velocity. Adapted with permission from Frank L. Rice, PhD, Integrated Tissue Dynamics, LLC.

investigators, especially those in manual medicine fields. Most LDF studies of manual procedures involve mobilization of the participants and taped probes, so investigators must manually select a portion of the LDF signal that is free from motion artifacts.[3] Therefore, reported changes in SBF during manual procedures have been challenged because complete signals cannot be extracted and analyzed, and those signals are needed for frequency-domain analysis.

Challenges to LDF Data Analysis: Time Domain vs Frequency Domain

To our knowledge, 1 research team has used a frequency-domain analysis to evaluate changes in SBF induced by cranial manipulation.[7,10] This team started to use a power spectral analysis of LDF recordings as used in a seminal

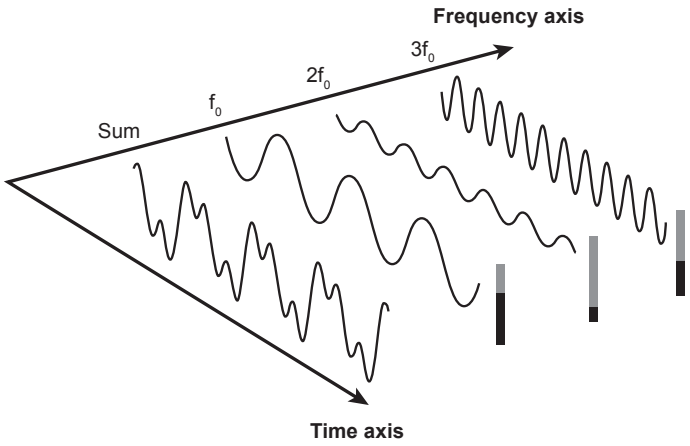

Figure 2.
Filtered laser Doppler flowmetry (LDF) signal expressed in a time domain and a frequency domain. The time-domain graph shows how the original LDF signal, labeled Sum, changes over time in its component frequencies. The fundamental frequency is shown at the frequency f_0, the second harmonic at frequency $2f_0$, and the third harmonic at frequency $3f_0$. In the frequency domain, the sine waves that add to form the complex time-domain signal can be separated conceptually. The frequency-domain graph shows how much of the LDF signal lies within each given frequency band over a range of frequencies. Reproduced with permission from the National Instruments Corporation.

study demonstrating that the palpable rhythm of the cranial rhythmic impulse (CRI) was synchronous with the 0.1-Hz frequency component of SBF recorded at the left earlobe.[6] Sergueef et al[10] used a Fourier-transform analysis to interpret changes in the LDF signal for 3 principal spectral peaks: the thermal or Mayer wave (1.2-5.4 cycles per minute), the baro or Traube-Hering wave (6.0-10.0 cycles per minute), and the respiratory wave (data not reported). Cranial manipulation, consisting of equilibration of the global cranial motion pattern and the craniocervical junction, increased the Traube-Hering wave and decreased the Mayer wave components, suggesting an influence on PSNS.[10] The application of a "compression of the fourth ventricle" procedure resulted in an amplification of the 0.1-Hz frequency oscillation of the LDF signal, which has been linked to the palpable phenomenon of the CRI.[7] Palpation over bony structures, such as the forehead,[7] might be different from palpation of soft structures, such as the earlobe,[6,8,10] and may not reflect the palpatory findings clinicians usually associate with the CRI. However, this research team seemed more interested in evaluating changes in specific spectra rather than overall changes in SBF associated with cranial procedures; therefore, they interpreted those changes as a possible influence over PSNS function. Further, they described the Traube-Hering-Mayer waves but not the usual 5 spectra of the LDF signal in the frequency domain.[32] They also did not describe the physiology of the SSNA, and they referred to the literature on muscle sympathetic activity and PSNS regulation of the heart to describe a putative role of cranial manipulation over PSNS activity.[7,10]

Two methods of expressing and analyzing SBF data in the time domain include the maximal effect method (maximum increase or decrease) and the integral measurement method. The former method is expressed as a percentage of the mean level of APU before stimulation.[9,11] With the latter method, APU data are averaged over different lengths of time—30 seconds[4] or 2 minutes[5]—and the experimental and final rest period values are converted into percent change from baseline. Analysis of variance methods are then used to evaluate changes in SBF associated with SMM. Because of the variability of SBF recordings, the maximal effect method is probably not appropriate, and the integral measurement method, which has been used to describe changes in other SBF indexes,[36] should be recommended. Results of a reactivity test may be expressed as raw APU or cutaneous vascular conductance (APU divided by mean arterial pressure), as the variation from baseline SBF (percent changes from baseline or changes in the area under the curve), or as a percentage of maximal vasodilation. The time over which SBF is averaged should be carefully chosen because it will influence data expression.[13] This period, however, has yet to be defined by investigators in manual medicine.

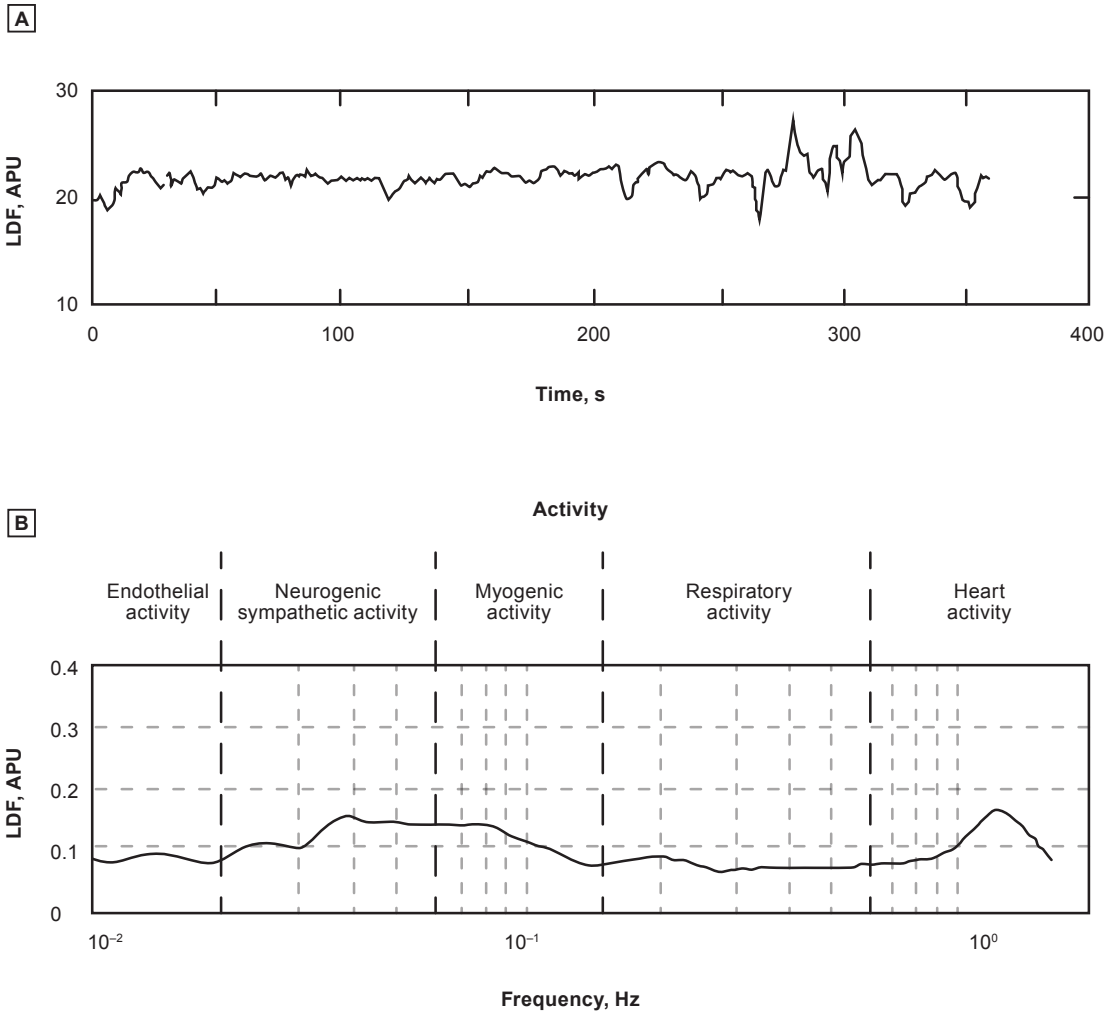

Figure 3.
A typical laser Doppler flowmetry (LDF) signal (A) and an example of transforming an LDF signal to a frequency domain (B). Because the time and frequency domains are equivalent, the 2 domains can be transformed into each other with the use of frequency-domain algorithms. The Fourier transformation and the inverse Fourier transformation, respectively, are typically used. The Fourier transformation is used to convert a signal of any shape into a sum of an infinite number of sinusoidal waves. The wavelet transform technique is another method used to analyze oscillation in the LDF signal.[32] *Abbreviation:* APU, arbitrary perfusion units. Adapted with permission from Huang et al.[32]

Discussion

The use of a time-domain or frequency-domain analysis may depend on the research question of the given study. Overall changes in SBF, for example, would require a time-domain analysis, and changes in PSNS function would require a frequency-domain analysis. Specific changes that may occur in 1 spectrum as detected with a frequency-domain analysis might be missed if investigators use only a time-domain analysis. The 2 types of analysis would then theoretically give a better description of OMT-induced physiologic changes. To date, investigators have used just 1 of these 2 methods. However, no consensus on the best way to express LDF data has been reached.[13]

Overall changes in LDF recordings have been studied in therapeutic procedures in other fields. A decrease in musculoskeletal pain has been associated with a short-term increase in SBF as measured with LDF after acupuncture,[37] interferential therapy,[38] ultrasound therapy,[39] and massage.[40] All of these procedures were applied locally, and mechanisms underlying initial local increase in SBF might have been different from remote changes occurring in the limbs after SMM. Furthermore, there seems to be a lack of information available to describe the potential link between changes in SBF and similar changes in the blood circulation in deeper tissues, which may have a greater clinical relevance for manual medicine practitioners (eg, osteopathic physicians, foreign-trained osteopaths, chiropractors, physiotherapists). Abnormalities of SBF responses to an ischemic reactivity test have been associated with left ventricular hypertrophy and increased relative wall thickness of the left ventricle and may indicate a ubiquitous impairment of microvascular function.[41] Similarly, LDF equipment may have the potential to indicate changes occurring in deeper tissues after manual procedures, but more research is necessary to describe the physiologic mechanisms involved, the reactivity tests to elicit them, and the normative values of healthy and symptomatic populations.

Compared with other SBF indexes, one of the main advantages of LDF is the ability to combine its use with noninvasive reactivity tests, such as the cold pressor or inspiratory gasp tests, which specifically assess SSNA control of SBF (*Figure 4*).[42] In previous manual medicine research, SBF data were not compared with these kinds of positive controls, and different SMM methods were used on healthy and symptomatic participants,[3] thus limiting the overall generalizability of the results. Researchers in other disciplines have used positive controls. Kimura et al[43] used the inspiratory gasp test as a positive control to compare the magnitude of the SBF reduction with that after a glutamate injection into latent myofascial trigger points. The use of LDF with reactivity tests may also help investigators discuss different physiologic pathways involved in overall SBF regulation by specifically eliciting or suppressing one of these physiologic pathways. For example, Mohammadian et al,[5] investigating manipulation-induced analgesia, used a 20-minute application of capsaicin cream over the forearm as a positive control to induce cutaneous inflammatory reactions and thereby increase SBF. Because SBF was not affected by a single SMM application, the investigators attributed the hypoalgesic effects to central rather than peripheral mechanisms.[5] The importance of local factors in the self-regulation of SBF[44] (*Figure 4*) along with a low and transient influence of SSNA[45] on SBF regulation might challenge previous interpretations of physiologic changes associated with SMM, especially because those changes have been solely associated with changes in PSNS function. To overcome this challenge, investigators should collect at least 1 other set of data with a PSNS marker, such as heart rate variability,[3] to differentiate local endothelial mechanisms from PSNS mechanisms involved in the initial physiologic changes that occur after SMM.

Frequency-domain analysis of LDF recordings was performed by a single research team investigating physiologic changes associated with palpation of the CRI,[7,8] after a cranial technique applied to the craniocervical junction,[10] and after compression of the fourth ventricle.[7] Because these researchers studied cranial palpation[6] and cranial manipulation,[7,8,10] in which the practitioner's

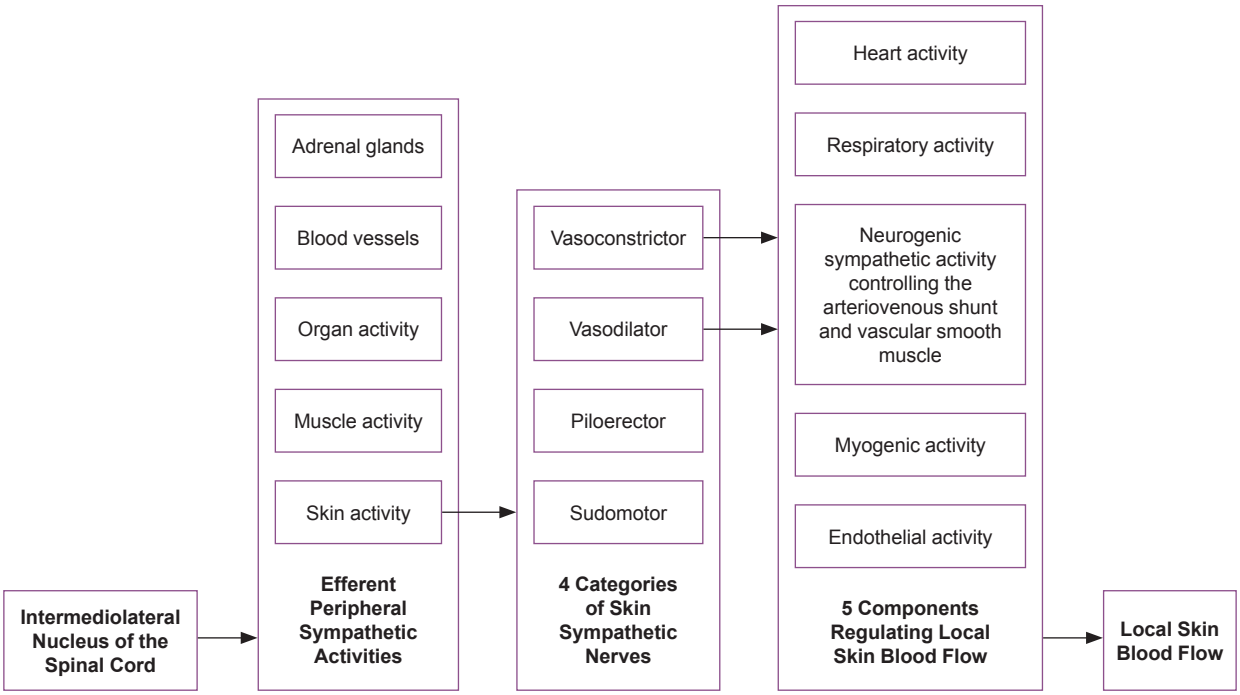

Figure 4.
Relationship between the overall efferent peripheral sympathetic nervous system, the 4 categories of skin sympathetic nerves, and the 5 components regulating local skin blood flow laser Doppler flowmetry signals record local variations in skin blood flow. These changes might not be a good marker of global peripheral efferent nervous system activity.

hands do not displace the participant's tissue, there would be limited movement of the probes taped over the earlobe or the forehead. The absence of motion artifacts is essential to perform a frequency-domain analysis of LDF recordings. The movement of probes taped to the lower or upper limbs after spinal manipulation,[4,5] spinal mobilization,[11] and peripheral joint mobilization[9] is inevitable, however, and a frequency domain analysis would not be suitable in those studies.

The hypothesis of venous vasomotion to explain rhythmic palpatory changes associated with CRI was first proposed by Farasyn.[46] Further, LDF equipment played a key role in evaluating this hypothesis and allowed Nelson et al[7,8] to show a link between the 0.1-Hz frequency component of SBF waves and palpation of the CRI. Fourier transformation of LDF recordings showed that some physiologic parameters may be affected by cranial manipulation; however, as stated by Nelson et al,[7] the therapeutic value of the changes is still unknown. The literature we reviewed did not discuss the specificities of SSNA over SBF regulation (*Figure 4*); therefore, we recommend a more prudent interpretation of previous results from LDF recordings that describe possible effects of cranial manipulation on PSNS function. Such claims might be supported by concomitant changes in other markers of PSNS function. Using heart rate variability might be useful,[3] but it requires a 5-minute recording,[47] and the pathways involved in the PSNS regulation of the heart and those involved in the SSNA of the head are likely different.

Conclusion

Laser Doppler flowmetry equipment is used in manual medicine research as a noninvasive tool to evaluate the overall SMM-induced changes in SBF, the correlation of the 0.1-Hz frequency component of SBF waves from palpation of the CRI at the head, and the influence of cranial manipulation on the 0.1-Hz frequency component of SBF waves. The use of the integral measurement method, where APU data are averaged over different lengths of time, should be preferred for time-domain analysis of LDF recordings. The use of LDF with noninvasive reactivity tests, such as the cold pressor or inspiratory gasp tests, might be useful to specifically assess SSNA and to compare the magnitude of changes with those after manual procedures. Because of the specificities of SSNA, however, LDF recordings of PSNS function, whether analyzed in the time or frequency domains, should be interpreted cautiously because SBF is probably not an appropriate marker for PSNS function. Because few studies exist for a meta-analysis to inform clinical practice, this review focused on what is currently known about LDF and SBF regulation. For future studies, the use of LDF equipment combined with markers of PSNS function or combined with reactivity tests involving specific physiologic pathways as positive controls may advance the usefulness of LDF in osteopathic manipulative medicine research.

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Author Contributions

Mr Zegarra-Parodi provided substantial contributions to conception and design, acquisition of data, and analysis and interpretation of data; Mr Zegarra-Parodi drafted the article; Dr Snider, Student Doctor Park, and Dr Degenhardt revised it critically for important intellectual content; and all authors gave final approval of the version of the article to be published.

References

1. Stark JE. An historical perspective on principles of osteopathy. *Int J Osteopath Med*. 2013;16(1):3-10. doi:10.1016/j.ijosm.2012.10.001.
2. Burns L. Viscerosomatic and somatovisceral spinal reflexes. *J Am Osteopath Assoc*. 1907;7(2):51-57.
3. Bolton PS, Budgell B. Visceral responses to spinal manipulation. *J Electromyogr Kinesiol*. 2012;22(5):777-784. doi:10.1016/j.jelekin.2012.02.016.
4. Karason AB, Drysdale IP. Somatovisceral response following osteopathic HVLAT: a pilot study on the effect of unilateral lumbosacral high-velocity low-amplitude thrust technique on the cutaneous blood flow in the lower limb. *J Manipulative Physiol Ther*. 2003;26(4):220-225.
5. Mohammadian P, Gonsalves A, Tsai C, Hummel T, Carpenter T. Areas of capsaicin-induced secondary hyperalgesia and allodynia are reduced by a single chiropractic adjustment: a preliminary study. *J Manipulative Physiol Ther*. 2004;27(6):381-387.
6. Nelson KE, Sergueef N, Glonek T. Recording the rate of the cranial rhythmic impulse. *J Am Osteopath Assoc*. 2006;106(6):337-341.
7. Nelson KE, Sergueef N, Glonek T. The effect of an alternative medical procedure upon low-frequency oscillations in cutaneous blood flow velocity. *J Manipulative Physiol Ther*. 2006;29(8):626-636.
8. Nelson KE, Sergueef N, Lipinski CM, Chapman AR, Glonek T. Cranial rhythmic impulse related to the Traube-Hering-Mayer oscillation: comparing laser-Doppler flowmetry and palpation. *J Am Osteopath Assoc*. 2001;101(3):163-173.
9. Paungmali A, O'Leary S, Souvlis T, Vicenzino B. Hypoalgesic and sympathoexcitatory effects of mobilization with movement for lateral epicondylalgia. *Phys Ther*. 2003;83(4):374-383.
10. Sergueef N, Nelson KE, Glonek T. The effect of cranial manipulation on the Traube-Hering-Mayer oscillation as measured by laser-Doppler flowmetry. *Altern Ther Health Med*. 2002;8(6):74-76.
11. Vicenzino B, Collins D, Benson H, Wright A. An investigation of the interrelationship between manipulative therapy-induced hypoalgesia and sympathoexcitation. *J Manipulative Physiol Ther*. 1998;21(7):448-453.
12. Roustit M, Cracowski JL. Assessment of endothelial and neurovascular function in human skin microcirculation. *Trends Pharmacol Sci*. 2013;34(7):373-384. doi:10.1016/j.tips.2013.05.007.
13. Stefanovska A, Sheppard LW, Stankovski T, McClintock PV. Reproducibility of LDF blood flow measurements: dynamical characterization versus averaging. *Microvasc Res*. 2011;82(3):274-276. doi:10.1016/j.mvr.2011.08.009.
14. Roustit M, Cracowski JL. Non-invasive assessment of skin microvascular function in humans: an insight into methods. *Microcirculation*. 2012;19(1):47-64. doi:10.1111/j.1549-8719.2011.00129.x.
15. Wright HM. The origins and manifestations of local vasomotor disturbances. *J Am Osteopath Assoc*. 1956;56(4):217-224.
16. Wright HM. Measurement of the cutaneous circulation. *J Appl Physiol*. 1965;20(4):696-702.
17. Beal MB, ed. *Selected Papers of John Stedman Denslow, DO*. Indianapolis, IN: American Academy of Osteopathy; 1993.

18. King HH, ed. *The Collected Papers of Irvin M. Korr*. Indianapolis, IN: American Academy of Osteopathy; 1997.

19. Beal MC. Viscerosomatic reflexes: a review. *J Am Osteopath Assoc*. 1985;85(12):786-801.

20. Bialosky JE, Bishop MD, Price DD, Robinson ME, George SZ. The mechanisms of manual therapy in the treatment of musculoskeletal pain: a comprehensive model. *Man Ther*. 2009;14(5):531-538. doi:10.1016/j.math.2008.09.001.

21. Pickar JG. Neurophysiological effects of spinal manipulation [review]. *Spine J*. 2002;2(5):357-371.

22. Schmid A, Brunner F, Wright A, Bachmann LM. Paradigm shift in manual therapy? evidence for a central nervous system component in the response to passive cervical joint mobilisation. *Man Ther*. 2008;13(5):387-396. doi:10.1016/j.math.2007.12.007.

23. Jowsey P, Perry J. Sympathetic nervous system effects in the hands following a grade III postero-anterior rotatory mobilisation technique applied to T4: a randomised, placebo-controlled trial. *Man Ther*. 2010;15(3):248-253. doi:10.1016/j.math.2009.12.008.

24. Puhl AA, Injeyan HS. Short-term effects of manipulation to the upper thoracic spine of asymptomatic subjects on plasma concentrations of epinephrine and norepinephrine: a randomized and controlled observational study. *J Manipulative Physiol Ther*. 2012;35(3):209-215. doi:10.1016/j.jmpt.2012.01.012.

25. Moulson A, Watson T. A preliminary investigation into the relationship between cervical snags and sympathetic nervous system activity in the upper limbs of an asymptomatic population. *Man Ther*. 2006;11(3):214-224.

26. Chiu TW, Wright A. To compare the effects of different rates of application of a cervical mobilisation technique on sympathetic outflow to the upper limb in normal subjects. *Man Ther*. 1996;1(4):198-203.

27. Sterling M, Jull G, Wright A. Cervical mobilisation: concurrent effects on pain, sympathetic nervous system activity and motor activity. *Man Ther*. 2001;6(2):72-81.

28. Charkoudian N. Skin blood flow in adult human thermoregulation: how it works, when it does not, and why [review]. *Mayo Clin Proc*. 2003;78(5):603-612.

29. Cutler MJ, Holland BS, Stupski BA, Gamber RG, Smith ML. Cranial manipulation can alter sleep latency and sympathetic nerve activity in humans: a pilot study. *J Altern Complement Med*. 2005;11(1):103-108.

30. Vongsavan N, Matthews B. Some aspects of the use of laser Doppler flow meters for recording tissue blood flow. *Exp Physiol*. 1993;78(1):1-14.

31. Wilder-Smith EP, Fook-Chong S, Liurong L. Reflex vasoconstrictor responses of the healthy human fingertip skin: normal range, repeatability, and influencing factors. *Microvasc Res*. 2005;69(1-2):101-105.

32. Huang HW, Jou IM, Wang CK, Chen PY, Wang WC, Lin CC. Power spectral analyses of index finger skin blood perfusion in carpal tunnel syndrome and diabetic polyneuropathy. *Exp Diabetes Res*. 2011;2011:465910. doi:10.1155/2011/465910.

33. Sandby-Moller J, Poulsen T, Wulf HC. Epidermal thickness at different body sites: relationship to age, gender, pigmentation, blood content, skin type and smoking habits. *Acta Derm Venereol*. 2003;83(6):410-413.

34. Fredriksson I, Larsson M, Strömberg T. Measurement depth and volume in laser Doppler flowmetry. *Microvasc Res*. 2009;78(1):4-13. doi:10.1016/j.mvr.2009.02.008.

35. Yvonne-Tee GB, Rasool AH, Halim AS, Wong AR, Rahman AR. Method optimization on the use of postocclusive hyperemia model to assess microvascular function. *Clin Hemorheol Microcirc*. 2008;38(2):119-133.

36. Perry J, Green A, Singh S, Watson P. A preliminary investigation into the magnitude of effect of lumbar extension exercises and a segmental rotatory manipulation on sympathetic nervous system activity. *Man Ther*. 2011;16(2):190-195. doi:10.1016/j.math.2010.10.008.

37. Guangjun W, Yuying T, Shuyong J, Tao H, Weibo Z. Change of blood perfusion in Hegu acupoint after contralateral Hegu acupoint was stimulated. *J Altern Complement Med*. 2012;18(8):784-788. doi:10.1089/acm.2011.0440.

38. Noble JG, Henderson G, Cramp AF, Walsh DM, Lowe AS. The effect of interferential therapy upon cutaneous blood flow in humans. *Clin Physiol*. 2000;20(1):2-7.

39. Noble JG, Lee V, Griffith-Noble F. Therapeutic ultrasound: the effects upon cutaneous blood flow in humans. *Ultrasound Med Biol*. 2007;33(2):279-285.

40. Nielsen A, Knoblauch NT, Dobos GJ, Michalsen A, Kaptchuk TJ. The effect of Gua Sha treatment on the microcirculation of surface tissue: a pilot study in healthy subjects. *Explore (NY)*. 2007;3(5):456-466.

41. Strain WD, Chaturvedi N, Hughes A, et al. Associations between cardiac target organ damage and microvascular dysfunction: the role of blood pressure. *J Hypertens*. 2010;28(5):952-958. doi:10.1097/HJH.0b013e328336ad6c.

42. Netten PM, Wollersheim H, van den Broek P, van der Heijden HF, Thien T. Evaluation of two sympathetic cutaneous vasomotor reflexes using laser Doppler fluxmetry. *Int J Microcirc Clin Exp*. 1996;16(3):124-128.

43. Kimura Y, Ge HY, Zhang Y, Kimura M, Sumikura H, Arendt-Nielsen L. Evaluation of sympathetic vasoconstrictor response following nociceptive stimulation of latent myofascial trigger points in humans. *Acta Physiol (Oxf)*. 2009;196(4):411-417. doi:10.1111/j.1748-1716.2009.01960.x.

44. Stefanovska A, Bracic M, Kvernmo HD. Wavelet analysis of oscillations in the peripheral blood circulation measured by laser Doppler technique. *IEEE Trans Biomed Eng*. 1999;46(10):1230-1239.

45. Krupatkin AI. Cardiac and respiratory oscillations of the blood flow in microvessels of the human skin [article in Russian]. *Fiziol Cheloveka*. 2008;34(3):70-76.

46. Farasyn A. New hypothesis for the origin of cranio-sacral motion. *J Bodyw Mov Ther*. 1999;3(4):229-237.

47. Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology. Heart rate variability: standards of measurement, physiological interpretation and clinical use. *Circulation*. 1996;93(5):1043-1065. doi:10.1161/01.CIR.93.5.1043.