Mucosal blood flow measurements using laser Doppler perfusion monitoring

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Abstract
Perfusion of individual tissues is a basic physiological process that is necessary to sustain oxygenation and nutrition at a cellular level. Ischemia, or the insufficiency of perfusion, is a common mechanism for tissue death or degeneration, and at a lower threshold, a mechanism for the generation of sensory signalling including pain. It is of considerable interest to study perfusion of peripheral abdominal tissues in a variety of circumstances. Microvascular disease of the abdominal organs has been implicated in the pathogenesis of a variety of disorders, including peptic ulcer disease, inflammatory bowel disease and chest pain. The basic principle of laser Doppler perfusion monitoring (LDPM) is to analyze changes in the spectrum of light reflected from tissues as a response to a beam of monochromatic laser light emitted. It reflects the total local microcirculatory blood perfusion, including perfusion in capillaries, arterioles, venules and shunts. During the last 20-25 years, numerous studies have been performed in different parts of the gastrointestinal (GI) tract using LDPM. In recent years we have developed a multi-modal catheter device which includes a laser Doppler probe, with the intent primarily to investigate patients suffering from functional chest pain of presumed oesophageal origin. Preliminary studies show the feasibility of incorporating LDPM into such catheters for performing physiological studies in the GI tract. LDPM has emerged as a research and clinical tool in preference to other methods; but, it is important to be aware of its limitations and account for them when reporting results.

INTRODUCTION
Circulation and perfusion of individual tissues is a basic physiological process that is necessary to sustain oxygenation and nutrition at a cellular level. Ischemia, or the insufficiency of perfusion, is a common mechanism for tissue death or degeneration, and at a lower threshold, a mechanism for the generation of sensory signals including pain. Ischemia is a common cause of pain from the myocardium in coronary heart disease, ranging from reversible changes in angina pectoris to acute myocardial infarction with its multitude of complications. Myocardial ischemia is usually due to stenoses of the larger epicardial arteries; but, microvascular changes, such as those commonly seen in patients with diabetes mellitus, can cause ischemia and biochemical changes triggering pain signalling from the tissues. Ischemia can similarly cause pain from abdominal organs, including the intestines, when stenoses of the proximal mesenteric arteries limit perfusion in the distal vascular bed. It is also possible that abnormalities in smaller vessels, and in their regulation of blood flow may...
cause similar biochemical changes in the intestinal wall and be an integral part of the pathogenesis of disease.

It is of considerable interest to study perfusion of peripheral abdominal tissues in a variety of circumstances. When studying the pathogenesis of various diseases, measurements of perfusion of the tissues affected may be essential to assess the relative contribution of ischemia to disease pathogenesis. Furthermore, in the surgical treatment of disease, assessment of perfusion may be important for assuring that anastomoses are established in well perfused segments of the gut. The beneficial or adverse effects of drug therapy might also be evaluated by monitoring perfusion of a segment of the gut.

MICROCIRCULATION IN THE GASTROINTESTINAL TRACT

Our knowledge of the peculiarities of the vascular bed of the gastrointestinal (GI) tract is still limited. There is considerable inter-individual variation in the anatomy of larger vessels, and the extent of collateral circulation, which may also explain differences in susceptibility to local ischemia. Perfusion is dependent on the arterial supply from the celiac, superior mesenteric and inferior mesenteric arteries. The watershed areas between these major arteries are likely to suffer from ischemia during acute or chronic arterial insufficiency.

The tissue volume occupied by moving blood cells is small; the average density of capillaries is about 50 capillaries per mm$^2$ of mucosal area, and on average 20% of capillaries are open under resting conditions, perfusion being mainly regulated by the opening and closing of precapillary sphincters. This autoregulation of perfusion is well established in several studies, including studies employing laser Doppler perfusion monitoring (LDPM) and is highly dependent on endothelial cell function.

MICROCIRCULATION AND THE SPECTRUM OF GASTROINTESTINAL DISEASE

Microvascular disease of the abdominal organs has been implicated in the pathogenesis of a variety of disorders, including peptic ulcer disease and inflammatory bowel disease (including both ulcerative colitis and Crohn's disease of the intestines). It has been suggested that apart from immunological and bacterial effects on the intestinal wall, changes in the microvasculature are essential for developing such key elements as mononuclear cell infiltration and fibrosis. Typically, the early stages of colitis show increased perfusion, whereas the later chronic stages of this disease show hypoperfusion of the mucosa. This was first shown in various animal models of acute inflammatory bowel disease (IBD), but also convincingly in patients with chronic disease with fibrosis. Importantly, it has been found that the capacity for vasodilatation is decreased in chronic IBD, suggesting a mechanism for ischemia and pain. It has been argued that in patients with Crohn's disease, chronic vascular changes may result in areas of microinfarction in the gut wall, leading to granulomatous inflammation and fibrosis.

LASER DOPPLER PERFUSION MONITORING (LDPM)

The basic principle of laser Doppler perfusion monitoring (LDPM; laser Doppler velocimetry, or laser Doppler flowmetry) is to analyse changes in the spectrum of light reflected from living tissues as a response to a beam of monochromatic laser light emitted (Figure 1). LDPM reflects the total local microcirculatory blood perfusion including perfusion in the capillaries (nutritive flow), arterioles (thermoregulatory flow - such as in the skin), venules and shunts (Figure 2).

One of the earliest papers concerning this issue was a report by Stern et al in 1975. They performed an ex-
periment to determine the feasibility of the method of coherent light scattering, in their case from a fingertip. They were able to demonstrate rapid microvascular reflexes which no other method was able to demonstrate at that time.

When a beam of light, carried by the fibre-optic probe, enters the tissues and hits moving blood cells in a random order, it undergoes changes in wavelength - a Doppler shift - while the wavelength of light hitting static tissue structures is unchanged. The magnitude and frequency distribution of these changes in wavelength are directly related to the number of moving blood cells, but relatively unrelated to their direction of movement.

The tissue volume occupied by moving blood cells is generally small; the average capillary density is about 50 capillaries per mm² mucosal area, and most photons do not undergo a frequency shift, but are backscattered or absorbed. The backscattered and Doppler broadened (extended) light carries information about the speed and concentration of blood cells traversing the scattering volume.

The quantity that is measured in LDPM is generally referred to as perfusion, and expressed in Perfusion Units (PU) which are arbitrarily chosen. In general it is not possible to change PU values into blood flow expressed as mL/min per g tissue; but, it can be done in specific preparations when calibration can be done. Perfusion is defined as the product of local velocity and concentration of blood cells. Speed refers only to the magnitude (mm/s) of the velocity vector, and even though the majority (99%) of blood cells in the undisturbed microcirculation are red cells, LDPM does not selectively measure red cells.

Penetration into the tissue explored depends on the wavelength of the emitted light, and is regulated by differences in fibre diameter/separation. Penetration depth is also influenced, to a great extent, by factors such as structure and density of the capillary bed. The measuring depth is often defined as the depth below the tissue to which approximately 2/3 of the surface light penetrates, and returns back to the tissue surface. A typical probe today is designed using a solid-state laser with a wavelength of 780 nm, one transmitting and one receiving fibre and a fibre separation of 0.25 mm. This could lead to a sample depth of about 0.5-1.0 mm, and the sample volume could be estimated to 1 mm³. This rather shallow measuring depth was the conclusion of several different studies published in the eighties; but, other studies executed during the same period suggested that LDPM had a capacity for transmural measuring in the GI tract. During the nineties a consensus was reached that LDPM monitors the microcirculation only in the mucosa and the upper submucosa of the GI tract.

Calibration generally has several purposes: to check the stability of the instrument; to establish the linearity of the instrument's response to blood flow; to establish a relationship between different instruments; and to relate the reading of the instrument to true perfusion, if possible. A gold standard for calibration of LDPM does not exist, and because the optical properties and distribution of blood vessels in the tissue are heterogeneous it is not realistic to calibrate the instrument to measure absolute blood flow. Therefore, the manufacturers of these instruments have provided a more simple calibration protocol, based on a two-point calibration, which makes it easy to calibrate the probes in a clinical or experimental situation. The motility standard is an aqueous suspension of polystyrene microspheres in Brownian motion. The method has some major shortcomings regarding its dynamic properties, and the suspension induces Doppler shifts which give rise to a homodyne measurement. However, in living tissues, the opposite situation is seen and a heterodyne spectrum is produced because the majority of photons do not undergo a Doppler shift.

During the early years of LDPM, the method was validated against well established methods for measuring blood flow, such as the electromagnetic method. However, this method clearly measures total blood flow, not just blood flow in the microcirculation. Later validation was performed using alternative methods known to selectively measure perfusion of the mucosal or muscularis layers i.e. local isotope washout, radioactive labelled microspheres, and H₂-clearance. Generally, it is not easy to evaluate these validation studies because the single point laser Doppler probe is measuring from a different and much smaller tissue volume. However, carefully executed experiments performed on preparations of canine stomach and intestinal wall showed excellent linear correlation between the LDPM signal obtained and total blood flow measured by the electromagnetic technique. One of these studies was also the first one to show that the gastric mucosa can autoregulate its blood flow, independent of other layers of the wall.

LDPM has emerged as a research and clinical tool in the absence of other methods, because it is a continuous, non-invasive and real time method for measuring microvascular blood flow, and it is also sensitive for detecting rapid changes in perfusion in the capillary circulation. LDPM is easily used in the clinical setting; but, to do so, one must be aware of its limitations. It is very important to ensure that the normal action and physiological responses of the microcirculation are not ignored when using this method. To get an optimal result there are both environmental and physical factors to take into consideration. These should be limited or accounted for when doing an investigation, in order to obtain reproducible data. It is also important to realise that it is impossible to say what the exact blood flow for any tissue is, and to remember that the optical properties and microvascular architecture cannot be determined in advance.

Physiological factors to be considered are temperature (thermoregulation has a significant effect on the microcirculation), the position and motion of the probe relative to the tissue surface, anatomical site and mental stress. Food and drugs also have effects on the microcirculation.

Technical limitations such as motion artifacts, multiple sequential Doppler shifts, variations in the specification of instruments from different manufacturers, lack
of exact knowledge of the depth of measurements, the instrument zero and/or biological zero[39] all have to be taken into consideration when analysing an investigation. There are several review articles published in recent literature describing these phenomena in more detail[30-33].

The laser Doppler probe is a sensitive motion detector, and many extraneous sources of noise e.g. respiratory movements cause mechanical vibrations in the same frequency range as the laser Doppler shifts produced by moving cells in the tissues (mucosa). Muscle fasciculation, vasomotion, respiration or any tissue movement relative to the laser Doppler probe may add noise to the laser Doppler signal. The GI organs are inherently motile, and motility-induced artefacts always occur during LDPM. One could argue that it is just noise which is recorded from the GI tract; but, Kiel et al[34] showed that this is not the case, and that true, perfusion can be measured from the GI tract.

Currently available instruments for LDPM generally also measure and display total backscattered light, of which Doppler shifted light makes up just a small fraction. The unit of measurement of backscattered light is mEV, whereas that of Doppler shifted light is mEV. The significance of total backscattered light is that when this is detected as stable; it is an indication of minimal motion artefacts between probe and tissue. One can argue that only when backscatter is stable can we assume that LDPM actually measures perfusion in the adjacent tissues and is not simply dominated by artefacts.

THE APPLICATION OF LASER DOPPLER PERFUSION MONITORING TO STUDY DISEASE PROCESSES IN GASTROINTESTINAL TISSUES

During the last 20-25 years numerous studies have been done in different parts of the GI tract using LDPM. The majority of studies have been done on animals or humans during anaesthesia or surgery. This gives much better control of factors which potentially might influence the measurements. In a fully awake human, it is much more complicated to do LDPM, especially in the upper GI tract. A survey of the literature indicates that research employing LDPM has focussed on a limited number of questions, primarily those evaluating the influence of drugs or surgical procedures on mucosal perfusion, especially in the upper GI tract[23] or cardiovascular system[24], and the influence of septic shock[25], portal hypertensive gastropathy[26,27], or hepatic cirrhosis[28,29]. LDPM has certainly been used in some other clinical settings, but less systematically.

During recent years, we have been working with a multi-modal device (Figure 3) incorporating a laser Doppler probe, developing this device primarily in order to investigate patients suffering from functional chest pain of presumed oesophageal origin[20], an illness which is incompletely understood. Distending a bag in the oesophageal body typically reproduces the painful sensa-

![Figure 3 The multi-lumen PVC catheter (Outer Diameter = 6.0 mm) and a distal bag for acoustic coupling and symptom provocation. A water perfused manometric system measures pressures inside the bag (BP) and proximal to the bag at locations P1 and P2. The end of the multi-lumen catheter was attached to a fenestrated cone of polyethylene. The distal end of the cone was attached to a smaller end mounted catheter (anchoring-tube) for distal attachment to the bag. A 20 MHz ultrasound probe was placed in the centre of the bag and the transducer of the laser Doppler probe (760 nm) was fixed with double-sided tape to the inner surface of the bag. Modified from Hoff et al[30], 2006.](image-url)
Laser Doppler perfusion monitoring has emerged as a research and clinical tool in preference to other methods because it is non-invasive, and yields continuous and real-time measurements of microvascular blood flow. Furthermore, it is sensitive to rapid changes in perfusion in the capillary circulation. LDPM is easily used in the clinical setting but, users have to be aware of its limitations and account for them when reporting results. LDPM can be included in multimodal devices, and we have demonstrated that simultaneous measurements of pressure, perfusion and ultrasound can be obtained from the oesophagus, when combined with bag distension. The quality of the data indicates that new insights can be obtained from studies in healthy volunteers and patients with functional chest pain. There are still some major challenges to face due to the fact that the method is highly motion-sensitive, and we cannot give the exact depth location from where these changes occur due to the fact that the method is highly motion-sensitive, and we cannot give the exact depth location from where these changes occur. Furthermore, it is difficult to obtain reliable flow data from the entire human oesophageal wall, and LDPM seems to be the best available choice, particularly for the multimodal device.

CONCLUSION

Future studies to look into ischemic- or strain-dependent pain mechanisms, may need to employ advanced distension protocols such as strain softening protocols.

REFERENCES

1. Gregersen H, Christensen J. Mechanically restricted regional blood flow might explain gastrointestinal pain. *Nat Clin Pract Gastroenterol Hepatol* 2005; 2: 378-379
2. Deban L, Correale C, Vetrona S, Malesci A, Danese S. Multiple pathogenic roles of microvasculature in inflammatory bowel disease: a Jack of all trades. *Am J Pathol* 2008; 172: 1457-1466
3. Hatoun OA, Miura H, Binion DG. The vascular contribution in the pathogenesis of inflammatory bowel disease. *Am J Physiol Heart Circ Physiol* 2003; 285: H1791-H1796
4. Wakefield AJ, Sankey EA, Dhillon AP, Sawyer AM, More L, Sim R, Pittilo RM, Bowles PM, Hudson M, Lewis AA. Granulomatous vasculitis in Crohn’s disease. *Gastroenterology* 1991, 100: 1279-1287
5. Stern MD. In vivo evaluation of microcirculation by coherent light scattering. *Nature* 1975; 254: 56-58
6. Doppler JC. Über das farbige Licht der Doppelsterne und einiger anderer Gestirne des Himmels. In: Versuch einer das Bradley’sche aberrations-theorem als integrierrenden Theil in sich schliessenden allgemeiner Theorie. Prag: In Commission bei Borrosch & Andre, 1842: 465-466
7. Nilsson GE. Perimed’s LDV flowmeter. In: Shepherd AP, Öberg PÅ, editors. Laser Doppler Blood Flowmetry, Hingham, Boston: Kluwer, 1990; 57-72
8. Leahy MJ, de Mul FF, Nilsson GE, Maniowski R. Principles and practice of the laser-Doppler perfusion technique. *Technol Health Care* 1999; 7: 143-162
9. Shepherd AP, Riedel GL. Continuous measurement of intestinal mucosal blood flow by laser-Doppler velocimetry. *Am J Physiol* 1982; 242: G668-G672
10. Kiel JW, Riedel GL, DiResta GR, Shepherd AP. Gastric mucosal blood flow measured by laser-Doppler velocimetry. *Am J Physiol* 1985; 249: G539-G545
11. Kvietsys PR, Shepherd AP, Granger DN. Laser-Doppler, H2 clearance, and microsphere estimates of mucosal blood flow. *Am J Physiol* 1985; 249: G221-G227
12. DiResta GR, Kiel JW, Riedel GL, Kaplan P, Shepherd AP. Hybrid blood flow probe for simultaneous H2 clearance and laser-Doppler velocimetry. *Am J Physiol* 1987; 253: G573-G581
13. Gana TJ, Huhlewych R, Koo J. Focal gastric mucosal blood flow by laser-Doppler and hydrogen gas clearance: a comparative study. *J Surg Res* 1987; 43: 337-343
14. Ahn H, Lindhagen J, Nilsson GE, Salerud EG, Jodal M, Lundgren O. Evaluation of laser Doppler flowmetry in the assessment of intestinal blood flow in cat. *Gastroenterology* 1985; 88: 951-957
15. Ahn H, Lindhagen J, Nilsson GE, Oberg PA, Lundgren O. Assessment of blood flow in the small intestine with laser Doppler flowmetry. *Scand J Gastroenterol* 1986; 21: 863-870
16. Johansson K, Ahn H, Lindhagen J, Lundgren O. Tissue penetration and measuring depth of laser Doppler flowmetry in the gastrointestinal application. *Scand J Gastroenterol* 1987; 22: 1081-1088
17. Ahn H, Lindhagen J, Lundgren O. Measurement of colonic blood flow with laser Doppler flowmetry. *Scand J Gastroenterol* 1986; 21: 871-880
18. Shepherd AP, Riedel GL, Kiel JW, Haumschild DJ, Maxwell LC. Evaluation of an infrared laser-Doppler blood flowmeter. *Am J Physiol* 1987; 252: G532-G539
19. Kernick DP, Tookie JE, Shore AC. The biological zero signal in laser Doppler fluximetry - origins and practical implications. *Pflugers Arch* 1999; 437: 624-631
20. Rajan V, Varghese B, van Leeuwen TG, Steenbergen W. Review of methodological developments in laser Doppler flowmetry. *Lasers Med Sci* 2008; Epub ahead of print
21. Humeau A, Steenbergen W, Nilsson H, Stromberg T. Laser Doppler perfusion monitoring and imaging: novel approaches. *Med Biol Eng Comput* 2007; 45: 421-435
22. Al-Rawi OY, Penfader SH, Page RD, Dave I, Russell GN. The effect of thoracic epidural bupivacaine and an intravenous adrenaline infusion on gastric tube blood flow during esophagectomy. *Anesth Analg* 2008; 106: 884-887, table of contents
23. Nygren A, Thoren A, Ricksten SE. Effects of norepinephrine alone and norepinephrine plus dopamine on human intestinal mucosal perfusion. *Intensive Care Med* 2003; 29: 1322-1328
24. van Haren FM, Sleight JW, Pickkers P, Van der Hoeven JG. Gastrointestinal perfusion in septic shock. *Anaeseth Intensive Care* 2007; 35: 679-694
25. Clarke DL, McKee A, Thomson SR. Octreotide lowers gastric mucosal blood flow in normal and portal hypertensive stomachs. *Surg Endosc* 2003; 17: 1570-1572
26. Mezawa S, Homma H, Ohta H, Masuko E, Doi T, Miyanishi...
K, Takada K, Kukitsu T, Sato T, Niitsu Y. Effect of transjugular intrahepatic portosystemic shunt formation on portal hypertensive gastropathy and gastric circulation. *Am J Gastroenterol* 2001; 96: 1155-1159

27 Taranto D, Leonardo G, Beneduce F, Vitale LM, Loguercio C, Del Guercio R, Del Vecchio Blanco C. Focal gastric blood perfusion in relation with the endoscopic signs and liver function in cirrhotic patients. *Digestion* 1997; 58: 58-63

28 Cirera I, Panes J, Bordas JM, Llach J, Bosch J, Pique JM, Teres J, Rodes J. Anemia increases gastric blood flow in noncirrhotic and cirrhotic patients. *Gastrointest Endosc* 1995; 42: 403-407

29 Clouse RE, Richter JE, Heading RC, Janssens J, Wilson JA. Functional esophageal disorders. *Gut* 1999; 45 Suppl 2: I131-I136

30 Takeda T, Nabae T, Kassab G, Liu J, Mittal RK. Oesophageal wall stretch: the stimulus for distension induced oesophageal sensation. *Neuрогastroenterol Motil* 2004; 16: 721-728

31 Richter JE, Barish CF, Castell DO. Abnormal sensory perception in patients with esophageal chest pain. *Gastroenterology* 1986; 91: 845-852

32 Drewes AM, Schipper KP, Dimcevski G, Petersen P, Anderson OK, Gregersen H, Arendt-Nielsen L. Multimodal assessment of pain in the esophagus: a new experimental model. *Am J Physiol Gastrointest Liver Physiol* 2002; 283: G95-G103

33 Barlow JD, Gregersen H, Thompson DG. Identification of the biomechanical factors associated with the perception of distension in the human esophagus. *Am J Physiol Gastrointest Liver Physiol* 2002; 282: G683-G689

34 Hoff DA, Gregersen H, Odegaard S, Nesje LB, Oevrboe K, Hausken T, Gilja OH, Matre K, Hatlebakk JG. A multimodal laser Doppler and endosonographic distension device for studying mechanosensation and mucosal blood flow in the oesophagus. *Neurogastroenterol Motil* 2006; 18: 243-248

35 Liao D, Zhao J, Fan Y, Gregersen H. Two-layered quasi-3D finite element model of the oesophagus. *Med Eng Phys* 2004; 26: 535-543

S- Editor Li LF L- Editor O'Neill M E- Editor Ma WH