Chapter 2. Background: Opioids and the Airways

2.1 The Problem: Opioid-induced respiratory depression

Opioids are important and commonly used drugs for post-operative pain relief in the hospital setting. They have a long history of medical use, and their general effects on the body are well-known. Their effective use for reducing post-operative pain is limited by concerns of respiratory depression which may be life-threatening.

2.1.1 Opioid usage

Morphine is the analgesic commonly used for pain relief and anaesthesia in the clinical setting. Morphine is a pure alkaloid, isolated from the milky exudate from the opium poppy plant (*Papaver somniferum*) [2, 4]. It has been exploited for at least 4000 years [5] for its antidiarrhoeal, antitussic, and analgesic properties (Table 2-1).

Table 2-1 Clinical uses of morphine [2]

Clinical use	Description
Analgesic	Opioid analgesics are most effective in the management of dull, diffuse, continuous pain, with adequate doses relieving even sharp, localised, intermittent pain. The pain relief is often accompanied by drowsiness, mental clouding, and an elevated mood (i.e. euphoria).
Antidiarrhoeal	Morphine delays gastric emptying, causing spasmodic increases in intestinal smooth muscle tone and a decrease in propulsive movements, thus allowing more time for the water to be resorbed from the intestinal contents.
Antitussive	Morphine acts as a cough suppressant. It is not usually used clinically for this purpose because of its side-effects and potential for abuse. Codeine and hydrocodone are the opioids preferred for this use, with reduced side-effects and less potential for abuse.

Anaesthesia

The term anaesthesia means literally “without feeling”. A general anaesthesia is more appropriately compared to a kind of coma (not sleep), one that is controllable and reversible.

The four components of anaesthesia are:

- Hypnosis or unconsciousness (a state of unawareness and unresponsiveness).
- Amnesia (lack of memory).
- Analgesia (lack of pain).
- Muscle relaxation.

Of the four anaesthesia components, opioids provide only analgesia [6].

Analgesic effect

Analgesia, or sedation, is described by the Task Force on Sedation and Analgesia [7] as a state that allows patients to tolerate unpleasant procedures while maintaining adequate cardiorespiratory function and the ability to respond purposefully to verbal command or tactile stimulation. It depresses a patient's awareness to the environment and reduces their responsiveness to external stimulation.

Opioids, such as meperidine, morphine, and fentanyl, are very effective pain killers (complete list in Table 2-2). They are not good at hypnosis or amnesia so for operative anaesthesia they need to be prescribed with other drugs [8].

Brody *et al.* [2] describes the pain experience as comprising transduction, transmission, perception and reaction. Opioid analgesics affect the transmission, perception and modify the reaction of pain [6].

Table 2-2 Opiates used for inducing analgesia or anaesthesia [9]

alfentanil	fentanyl	methadone	propoxyphene
Buprenorphine	heroin	morphine	Remifentanil
carfebtanil	hydrocodone	naloxone	sufentanil
codeine	hydromorphone	naltrexone	tilidine
dihydrocodeine	LAAM	beta-hydroxy-3-methyl fentanyl	tramadol
diprenorphine	levorphanol	oxycodone	enantiomers
etorphine	meperidine	oxymorphone	diastereomers

Opioid advantages

The advantage of using opioids for pain relief is their selective analgesic quality: they can provide profound analgesia with no effects on other sensory modalities (i.e. without losing unconsciousness) [3] and without affecting muscle activity [10].

Clinical use

Opioids are important analgesic drugs [11] that continue to be the mainstay of perioperative analgesia [12] and are regularly prescribed in hospital for pain relief [13]. Morphine remains a popular and rational choice for PCA therapy [6, 14] and chronic pain relief [15] and is by far the most commonly administered intrathecal opioid [11].

Peak effect

Morphine is used for post-surgical pain relief because although it is slow acting it has a long half-life; the onset is in 10-20 min (intravenous) or 30 min (epidural), the peak occurring at 15-30 min (intravenous) or 30-60 min (epidural) [16].

Fentanyl is a synthetic agent that is 100 times more potent than morphine [3] with a shorter action time; peak onset of respiratory depression is 5-10 min compared to morphine 30 ± 15 min [6]. It is given preoperatively and during induction of anaesthesia in the operating theatre for its short-lived, fast-acting sedative qualities which allow patients to tolerate unpleasant procedures by relieving anxiety, discomfort, or pain [7].

2.1.2 Problems with opioid use

While there are many clinical side effects with therapeutic doses of opioids (Table 2-3), there are two main problems that limit the clinical use for analgesia: dosage titration and respiratory depression [2, 17-20].

Table 2-3 Clinical problems with therapeutic doses of morphine [2]

Respiratory depression	Respiratory depression is the most serious side-effect and the principal cause of death from overdose. Opioids decrease the sensitivity of chemoreceptors in the brainstem to carbon dioxide, a normal stimulus of ventilatory reflexes. The result is a blunting of the ventilatory response to increases in the carbon dioxide tension (pCO_2) in blood and cerebrospinal fluid. Respiratory depression is shown by an elevation in the blood pCO_2. In clinical anaesthesia, depression of respiration, either the rate or tidal volume, or both, often serves as a useful index of the adequacy of pain relief.
Sedation	Drowsiness may need to be enhanced by concurrent administration of another sedative.
Constipation	Smooth muscle stimulation, but diminished propulsive peristalsis increases bowel transit time causing constipation.
Nausea	Stimulation of the chemoreceptor trigger zone in the area postrema commonly produces nausea, sometimes vomiting.
Endocrine	Lowers plasma concentrations of lutenising hormones (females) and of testosterone (males) leading to menstrual cycle irregularities and to male sex impotence.
Miosis	Pupillary constriction is used clinically to gauge adequacy of pain relief and pin-point pupils diagnose an overdose.
Cardio-vascular	This system is relatively unaffected by opioid analgesics. High doses of morphine may cause orthostatic hypotension. Peripheral resistance is decreased by dilation of arterioles and veins, and causes a decline in blood pressure, but this is not significant in supine patients.
Immuno-suppression	Animal studies show that opioid analgesics suppress the immune system.
Tolerance	With repeated drug administration, larger doses are necessary to produce the same response.
Physical dependence	Continuous exposure to opioid analgesic results in the development of physical dependence, a state in which the body has adapted to the presence of the drug and therefore requires the drug in order to function normally.
Euphoria	The abuse potential of morphine is high because of the subjective effect of euphoria, producing a positive mood with feelings of well-being. Proper medical use rarely leads to addiction, psychological dependency or subsequent drug abuse.

Dosage titration

Provision of effective opioid analgesia is difficult to determine as its effectiveness is dependent not only on the intensity of pain [3, 21], but also on a number of factors including the relative intensity of non-pain-related internal and external stimuli such as ambient noise, light, and patient discomfort (e.g. full bladder, trauma requiring nerve block). It cannot be correlated against patient weight as there is large dose variability between patients [22] though

there are indications that lean body mass may be a better predictor for dosage regimens [6].

If too much opioid is given, or the pain-related stimulation is reduced, sedation predominates and, if strong enough, can lead to loss of airway tone or respiratory depression or both, leading to hypoxia and brain injury [22].

Respiratory depression

Respiratory depression, or hypoventilation, is a decrease in the frequency of breathing. It is variously defined in the literature [23] depending on which of the multiple effects are being monitored. A range of definitions are provided in Table 2-4.

The initial effect is to reduce the rate and volume of breathing [5]; this can subsequently cause hypercapnia, an increase in carbon dioxide concentration in the blood, $PaCO_2$, and if severe can cause hypoxaemia, low concentration of oxygen in arterial blood, and then hypoxia, reduced tissue oxygen concentration [24]. Respiratory depression can also appear as upper airway obstruction [20] which can also result in hypoxaemia [25].

Table 2-4 Respiratory depression definitions

Definition	Author
Shallow respirations, irregular or periodic breathing, and RR $\leq$ 10 br·min^{-1}	[26]
Oxygen saturation < 90%, $ETCO_2$ > 50 mmHg, an absolute $ETCO_2$ change from baseline > 10 mmHg, or loss of $ETCO_2$ waveform	[27]
$SpO_2 \leq$ 92% or $ETCO_2$ change ≥ 10 mmHg from presedation baseline, or intrasedation $ETCO_2$ level $\leq$ 30 or ≥ 50 mmHg	[28]
RR < 10 br·min^{-1}, $ETCO_2$ > 60 mm Hg, apnoea > 30 s, SpO_2 < 90%, HR < 50 or > 120 bpm	[29]
Not defined in 46% of 96 studies using morphine for postoperative analgesia, 25% defined using RR (usually <10)	[23]
SpO_2 range < 85% to < 94%	[23]
Hypoxaemia: mild SpO_2 < 94%, severe SpO_2 < 85% for > 6 min·hr^{-1}	[19, 30-32]
$PaCO_2$ > 50 mmHg	[23]
Blood gas levels, ABG: PaO_2 < 60 mmHg or $PaCO_2$ > 50 mmHg	[6, 23]

Abbr: ABG, arterial blood gas; HR, heart rate; Pa, arterial pressure; RR, respiratory rate.

Respiratory failure is difficult to predict and can become life-threatening in a few minutes, but it can also build up gradually [5, 33]. Hypercapnia has been suggested as the most rigorous definition of respiratory depression for clinical use [23].

High doses of opioids usually eliminate spontaneous respirations without necessarily producing unconsciousness. Patients receiving high doses of opioids may still be responsive to verbal commands and often will breathe when directed to do so [6]. Opiates have the added effect of depressing the normal ventilatory response to hypercapnic and hypoxic events [6, 34].

Respiratory depressant effects can last longer than the analgesia [16]. The peak onset of respiratory depression after an analgesic dose of morphine is 30 ± 15 min, slower than after comparable doses of fentanyl, 5 to 10 min. Respiratory depression induced by small doses of morphine usually lasts longer than equipotent doses of fentanyl. Higher doses of fentanyl (50-100 $\mu g \cdot kg^{-1}$) can require ventilation for 6-18 hr to alleviate persistent respiratory depression [6]. Morphine (1-2.5 mg intrathecal) peak respiratory depression occurs between 5-10 hr and then slowly decreases to baseline levels by 20 hr [35].

The stimulation of pain is a “natural antagonist’ to respiratory depression by opioids and other sedative drugs. Respiratory depression may become evident after the pain stimulation ceases or decreases in intensity [36, 37]. The ability of pain to obviate the respiratory depressant effects of opioids has not been fully delineated [37].

Upper airway obstruction can quickly occur and lead to mortality with no, or small, non-sedative doses of opioid. The South Australian coroner [38] reported on a finding of death in 2002 by cardiorespiratory arrest of a 27 yr old man as a result of airway obstruction due to inflammation (sublingual and submandibular cellulitis) following molar tooth extraction. Within 30 min of receiving a non-sedative dose of morphine he was at the nursing station, distressed, with difficulty in breathing. The nurse followed him back to bed whereupon she heard stridor. While putting him onto the bed she noted his

pupils were fixed and dilated; there was no response to cardiopulmonary resuscitation.

2.1.3 Incidence of respiratory depression

Respiratory depression due to opioid use has been widely reported [7, 24, 32, 35, 39-43]; all currently available opioids used for analgesia suppress respiratory drive [20, 40].

Opioid-induced respiratory depression can be severe and life-threatening [11]. The incidence of post-operative patients who have severe complications from opioid administration varies 0.1-1% no matter what route of administration [6] (Table 2-5). The effect of these complications is usually severe resulting in morbidity or mortality [44].

Table 2-5 Incidence of severe respiratory depression complications with opioid administration

Incidence	Administration details	Author
0.03%	PCA and EOA	[45]
0.5%	PCA (N = 1600)	[43]
0.53%	Various APS (N = 3016)	[44]
0.75%	PCA and NCA	[46]
0.9%	Therapeutic doses opioids in hospitalised adults	[26]
0.1-1%	No matter what route of administration	[6]
0.04-1.2%	EOA vs. intravenous morphine for post-operative pain (N = 2696)	[47]
1.28%	PCA with background opioid infusion	[48]
0-1.3%	Respiratory depression in children	[49]
0.24-1.6%	Review EOA (N = 1014 to 1,304,214)	[50]
1.8%	Various APS (N = 2509)	[51]
0.2-1.9%	Epidural PCA	[4]
6%	Initial dose PCA with long-term transdermal fentanyl	[52]

Abbreviations: APS, Acute pain service; EOA, Epidural opiate analgesia; N, number of subjects; NCA, Nurse controlled anaesthesia; PCA Patient-controlled anaesthesia.

An evaluation of American Society of Anesthesiologists adverse anaesthetic outcomes by Bhananker *et al.* [36] found respiratory depression due to oversedation and a lack of vigilance the leading cause of serious patient injuries (21%, n = 25) in monitored anaesthesia care. The respiratory

depression caused by oversedation was due to an absolute or relative overdose of sedative-hypnotic-analgesic agents. Nearly half these claims were judged as preventable by better monitoring, including capnography, improved vigilance or audible alarms [36].

Respiratory depression definitions

The different definitions for respiratory depression, summarised in Table 2-6 are based on the method of measurement:

- Respiratory rate (RR)
  Measurement by clinical observations, ECG impedance changes with volume in lungs, spirometry changes in flow or pressure, or gas concentration changes (e.g. O_2 or CO_2).
- Hypercapnia
  End tidal carbon dioxide, $ETCO_2$, measured by capnometry (infrared sensor on airway gases), or arterial blood gas, $PaCO_2$.
- Hypoxaemia
  Decreased concentration of oxygen in arterial blood, oxygen saturation, SpO_2, measured by pulse oximetry (infrared sensor on finger), arterial blood gas, PaO_2.
- Ratio of inspiratory to expiratory time (I:E)
  Opioids prolong both inspiratory (30%) and expiratory time (95%) in the respiratory cycle, but increase the expiratory duration more (compared to other volatile anaesthetics that cause a reduction in tidal volume [5]). Fentanyl (0.3 $\mu g \cdot kg^{-1}$) does not influence tidal volume but decreases ventilation (28%) as a result of a decrease in inspiratory flow (10%) and increase in duration of expiration (45%) [53].

Table 2-6 Relationship between measurement method and respiratory depression definition

	RR	High CO_2	Low O_2	I:E
Observations	✓	×	×	×
Impedance (ECG)	✓	×	×	×
Spirometry	✓	×	×	✓
Airway gases	✓	✓$ETCO_2$	✓$ETCO_2$	✓
Capnography	✓	✓$ETCO_2$	×	✓
Arterial blood gases	×	✓ $PaCO_2$	✓ PaO_2	×
Pulse oximetry	×	×	✓ SpO_2	×

2.1.4 Reducing incidence of respiratory depression in the hospital

There are three main areas which have focussed on reducing the incidence of respiratory depression in the clinical environment: monitoring, clinical interventions, and monitoring opioid potency.

Monitoring respiratory depression

In critical care areas of the hospital, continual machine monitoring of physiological parameters is routine, respiratory depression is identified at an early stage and treated. In some situations, for example during operations, it is anticipated that respiratory depression will occur so the airway is supported (intubation) and mechanical ventilation is provided.

In other areas of the hospital (e.g. recovery, post-anaesthesia care unit, PACU high dependency unit, and trauma wards) continuous physiological monitoring is not the standard and is usually not necessary. In these areas, regular nursing observations monitor patient status, particularly for sedation level and respiratory status [26]. For example, standing observations with patient-controlled epidural anaesthesia are to check respiratory rate, level of consciousness, blood pressure and heart rate every 1 hr for 12 hr, then every 2 hr for 12 hr, and every 4 hr thereafter [4].

Adequate monitoring can detect respiratory slowing, the best indicator of early respiratory depression [54], long before noticeable respiratory depression with continuous opioid infusions [55]. Guidelines [7] advise that ventilatory function

can usually be effectively monitored by observation of spontaneous respiratory activity or auscultation of breathing sounds.

There are disadvantages of using respiratory rate alone as a measure of respiratory depression: respiratory rate does not correlate with hypoxaemia or depressed response to carbon dioxide (CO_2), and patients with normal respiratory rate may be hypoxaemic or hypercapnic [23]. If the respiratory depression is caused by obstructive apnoea (airway collapse), rather than central apnoea (loss of respiratory drive), respiratory rate measured by ECG impedance will continue to show false signs of adequate respiration levels [36] (with paradoxical chest movement [56]), though ventilation is impaired.

Pulse oximetry monitors are available in most areas of the hospital; however, the pulse oximeter will not detect a lack of oxygen in the blood for some minutes after breathing has stopped. It takes time for the reduced oxygen level in the lungs to affect the concentration held in the blood. This blood is then transported to the periphery where it will eventually be detected by the pulse oximeter. The overall time depends on the sensor location, the averaging time used in the monitor, and the alarm threshold. Provision of oxygen to patients with the risk of respiratory depression further reduces the likelihood of pulse oximetry to detect hypoxic events.

The use of spirometry or capnography with a facemask can detect these respiratory events and although not classed as invasive technologies, are not well tolerated by patients over the days of monitoring that would be required, are still expensive, and require expert clinical knowledge about both the information produced and technological problems with their use.

Other devices have been invented to monitor respiration at lower cost then spirometry or capnography but are not widely used having reliability problems: monitoring exhaled carbon CO_2 in patients spontaneously breathing O_2 through a clear plastic face mask [57]; a simple electronic flowmeter attached to a facemask using pyroelectric or piezoelectric sensor for flow [58]; respiR8 (Anaxsys, Surrey, UK) continuous rate respiratory monitor requires mask with in-built sensor; provision of O_2 through T-piece to observe mist formation

(FCOT Oxygen therapy kit, Flexicare Inc, Irvine, CA, USA). A pitot-tube flowmeter has also been developed for sleep apnoea use but also requires a facemask [59].

Flow has been monitored indirectly with external thermistors particularly for sleep apnoea, being less invasive and more tolerated over the longer term than a mask; they are appropriate for apnoea detection however not able to quantify reduced airflow because of their slow and nonlinear response [60, 61]. Multiple thermistors have been used to reduce the error rate when used for evaluating sleep apnoea, but they also have problems at low breathing rates [62]. More recently thermal imaging methods have been used to detect flow [63].

In practise, monitoring upper airway obstruction with indirect methods is difficult; one group have determined the minimum non-invasive requirements are for nasal pressure and airflow requiring a nasal mask pnuemotachograph [60].

Clinical interventions

There are a few methods used clinically to minimise the occurrence of respiratory depression: reduced usage of opioids, minimised dosage with monitored anaesthesia care, multimodal techniques with the use of adjuvants to reduce opioid dose, and administration of naloxone if reversal is required.

Monitored anaesthesia care or conscious sedation can be used for some minor clinical procedures to reduce the amount of sedation. In theory, the patient can maintain consciousness, and their airway, however this method is not without problems [22]. Opioid analgesics are being replaced with clonidine, dexmedetomidine or ketamine to reduce respiratory depression [64, 65]. This replacement does not extend to post-operative use.

Multimodal or balanced analgesia techniques for postoperative pain management have currently focused on the combination of an opioid with a non-opioid to reduce opioid-related side effects. This may eventually lead to more optimal pain relief in an opioid-free environment [65].

Monitoring opioid potency

No high-resolution measure of analgesia is available so opioid potencies are estimated by surrogate measures [6]:

- Reduction of the minimum alveolar concentration required to produce lack of movement to skin incision (not useful outside the operating room).
- The hypnotic effect of central nervous system depressants is able to be tracked by electroencephalogram, EEG, devices [66] with measures of median frequency, spectral edge (95% of spectral power below) and bispectral index [66-68].

These measures cannot asses the degree of analgesia or respiratory depression of analgesic doses of opioids [21, 22].

2.1.5 Summary

Opioids are commonly used for post-operative pain, the benefits far outweighing the known life threatening side-effect of respiratory depression that can occur in 0.1-1% patients. This respiratory depression is easily reversed if detected quickly. The respiratory depression can be detected by monitoring breathing rate regularly (but not continuously); however, current techniques for simple non-invasive monitoring are not effective at early detection of this problem. There is a need to detect critical respiratory events with a continuous non-invasive monitor that is well-tolerated by patients.

2.2 Opioid effect on respiration

Opioids produce their sedative effect by direct action on the central nervous system, transmitted to peripheral organs via the autonomic nervous system (ANS), decreasing sympathetic activity and increasing parasympathetic activity and vagal tone [6].

2.2.1 ANS

The autonomic nervous system automatically maintains the body's internal state: pupil size, salivation, digestion, respiration, heart rate, perspiration, urination. It is controlled by two paths; the parasympathetic system acts as a brake, controlling relaxation, digestion, conservation of energy and resources, and the sympathetic system, or accelerator, mobilises and expends energy, responds quickly to actions, from simple actions such as standing up, to the more complex and life-threatening fight or flight response to danger.

Parasympathetic activity via the vagus nerve normally stimulates gut activity (eating, digesting, and waste removal – excess causing diarrhoea), excites smooth muscle, and increases secretions (salivation, lacrimation, sweating and increased stomach acid production).

2.2.2 Opioid effect on ANS

Opioids main effects are to reduce sympathetic and enhance parasympathetic (including vagal) tone generally as described in Table 2-7 [6].

In reality, opioids have a mixture of effects as shown in Figure 2-1. The boxes on the left highlight enhanced parasympathetic activity effects, the right boxes show reduced parasympathetic or increased sympathetic effects.

Table 2-7 Opioid effects with nerve actions

Nerve action	Effect from morphine and other mu-receptor agonists such as fentanyl
Excitatory action on parasympathetic nerve:	Constriction of the pupil.† Increase feeding [6]. Decrease respiratory function.
Excitation of vagus:	Bradycardia by stimulation of central vagus nucleus and possibly blockade of sympathetic actions [6]. Vagotonic effects depress cardiac conduction; prolong the RR-interval and prolong AV node conduction [69].
Reduction of sympathetic tone:	Impairs certain sympathetic reflexes involving peripheral veins. Vasodilation after morphine. Peripheral vessel smooth muscle relaxation by fentanyl (dogs).
Reduced vagal or increased sympathetic tone	Depressant effect on mucociliary flow in trachea. Decrease in gastrointestinal motility (antidiarrhoeal effect), lower oesophageal sphincter relaxation, delay in gastric emptying, increase in gastric pH, increase biliary duct pressure and sphincter of Oddi tone (dose-dependent action).

† changes in pupil size are related to plasma opioid levels, however the change may be too small to be clinically useful in assessing the opioid effect [6].

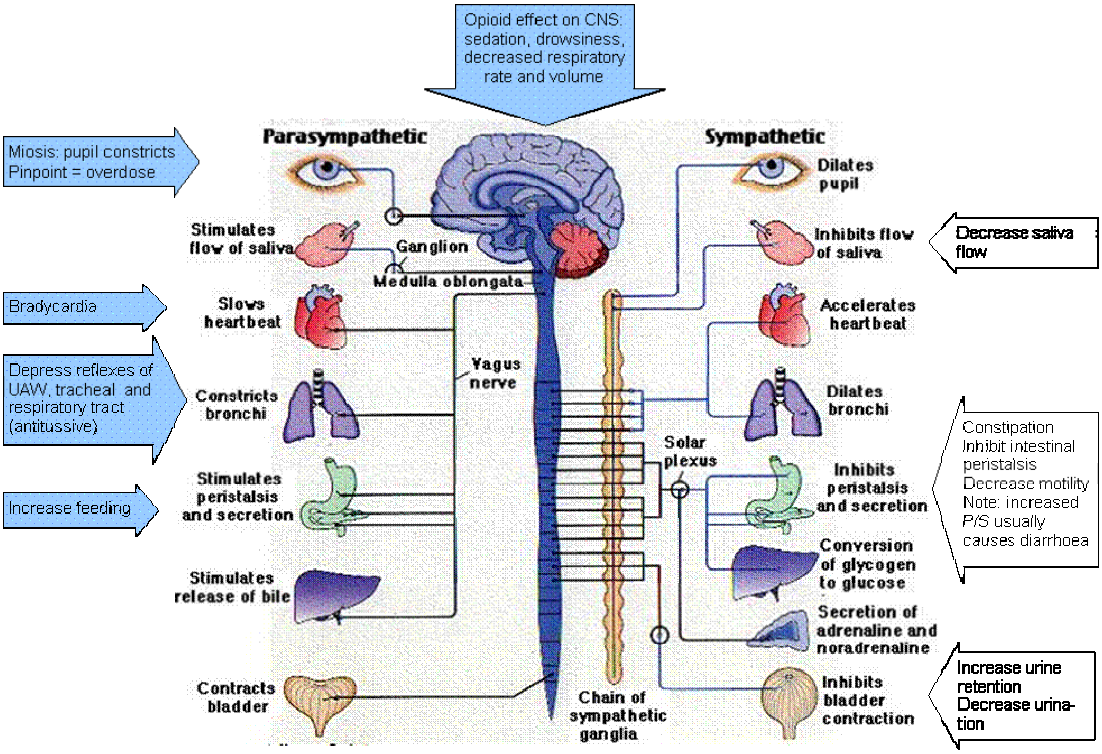


Figure 2-1 Summary of opioid effects on the autonomic nervous system: enhanced parasympathetic (left) and enhanced sympathetic actions (right) (modified from [70])

Morphine and the pure mu agonists, fentanyl and oxymorphone, are usually inhibitory; the mu-opioid receptor being the source of their antinociception, bradycardic, and respiratory depressant effects [71, 72].

The opioid effects of particular interest to this study are:

- Decreased respiratory function (central and peripheral effects).
- Bradycardia (increased vagal activity).

2.2.3 Multiple opioid respiratory effects

Opioids affect breathing and the respiratory system in a variety of ways (Table 2-8). Some of the opioid respiratory effects are not relevant to this study:

- Some effects are only seen with large doses of opioids: blunting of ventilatory response to high CO_2 and O_2, muscle and airway rigidity, and airway smooth muscle changes.
- Airway reflexes, such as laryngeal stimulation, usually cause vigorous reflex responses, such as expiration reflex spasmodic panting, cough reflex, and apnoea with laryngospasm[1]. Increasing doses of fentanyl (up to 200 µg) reduce the incidences of all these responses, in a dose-related manner, except for apnoea with laryngospasm [73]. The airway reflex is not relevant to this study.
- The opioid effect on arterial baroreflex sensitivity.

The opioid respiratory effects of interest to this study are on upper airway muscle tone, respiratory drive and rhythm. The mechanisms of these effects will be analysed in more detail after describing the airway muscles, their innervation and normal respiration.

[1] Sudden and uncontrollable closure of the larynx that blocks the passage of air to the lungs; often seen in anaphylactic reactions.

Table 2-8 Opioid effects on respiratory system and mechanism

Effect	Mechanism
Airway rigidity	Activation of central μ-receptors increases rigidity of the airway [74] and the respiratory muscles [6]. It occurs with loss of consciousness, may be associated with diminished ventilation, apnoea and vocal cord closure [16] and can be detected by paradoxical chest movement [56].
Arterial baroreflex sensitivity	Fentanyl modulates the respiratory frequency fluctuation of HRV, partly caused by the effects of fentanyl on arterial baroreflex sensitivity [75].
Muscle rigidity	Muscle rigidity has been well documented [18, 76]. Chest wall rigidity is mostly seen with rapid bolus doses rather than infusions [77, 78].
Respiratory drive and rhythm	Depressed brain centres (less responsive to electrical stimulation) where respiratory rhythms are generated disrupting the pattern of breathing [26]. Effect noticeable with low doses of opioids [79, 80]. Fentanyl slows respiratory rhythm [54, 81].
Response to high CO_2	Reduced hypercapnic response, responsiveness of CO_2 chemoreceptors in the medulla; increased blood levels of CO_2 do not produce an increase in respiratory rate. The body must rely on the less sensitive O_2 driven respiration regulating mechanism [26]. Additionally, apnoeic threshold and resting end-tidal PCO_2 are increased by opioids, and hypoxic ventilatory drive is decreased [6, 82, 83]. Effects on chemosensory pathways occur with greater fentanyl doses with only large doses reducing the response of ventilation to CO_2 [79].
Response to low O_2	Depressed ventilatory response to hypoxia by central (not peripheral) mediation [11]. The time course is similar to that of CO_2 response.
Upper airway muscle tone	Airway obstruction lies above trachea at the glottis (larynx vocal cords); glottic closure causing upper airway obstruction [84, 85].

2.2.4 Airway form and function

Lower airway

The lower airway, below the larynx (in the trachea, and bronchi greater than 1mm in diameter), is unable to collapse as it is supported by multiple cartilage rings [86]. The lower airway smooth muscle is under autonomic control with only sparse sympathetic innervation of the lower airway; contraction is due to cholinergic parasympathetic nerves and relaxation is due to non-cholinergic parasympathetic nerves [87].

Upper airway

The upper airway (Figure 2-2), from the nasopharynx to the larynx, is a collapsible tube supported by more than 20 pairs of muscles, most of which do not have respiration as their primary function [88]. They require a sophisticated motor control system to enable eating (mastication), swallowing

(deglutination), communication (speech and facial gestures), smelling (olfaction), and upper airway protection (sneezing, coughing or swallowing) [89, 90].

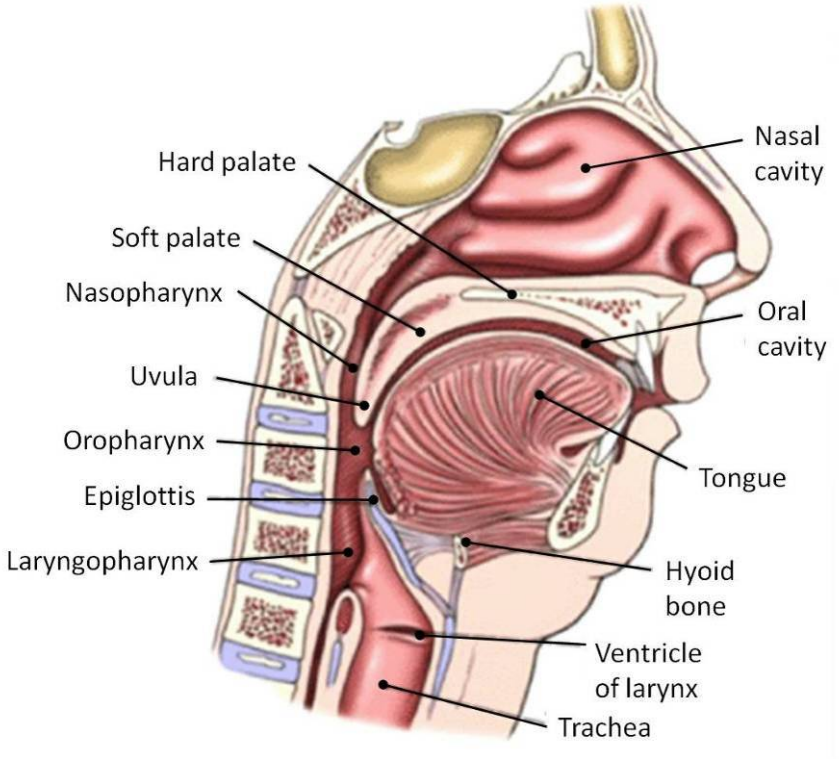


Figure 2-2 Upper airway with pharynx: nasopharynx, oropharynx and laryngopharynx (modified from [91])

Pharynx

The pharynx is the only portion of the large airways that lacks rigid support. The anterior wall is surrounded by soft tissue that tends to narrow and collapse the pharynx with the negative pressure generated during inspiration [92, 93]. Three parts of the pharynx can collapse: the retropalatal segment behind the soft palate, the retroglossal segment behind the tongue , and the retroepiglottic segment behind the epiglottis [34].

Upper airway patency

Maintaining the patency of the upper airway during inspiration (and swallowing) requires reciprocal activation of mechanically opposing dilator and constrictor muscles [82, 93]. The superior and middle pharyngeal constrictors (Figure 2-3 a) are phasically active during expiration [82].

a) b)

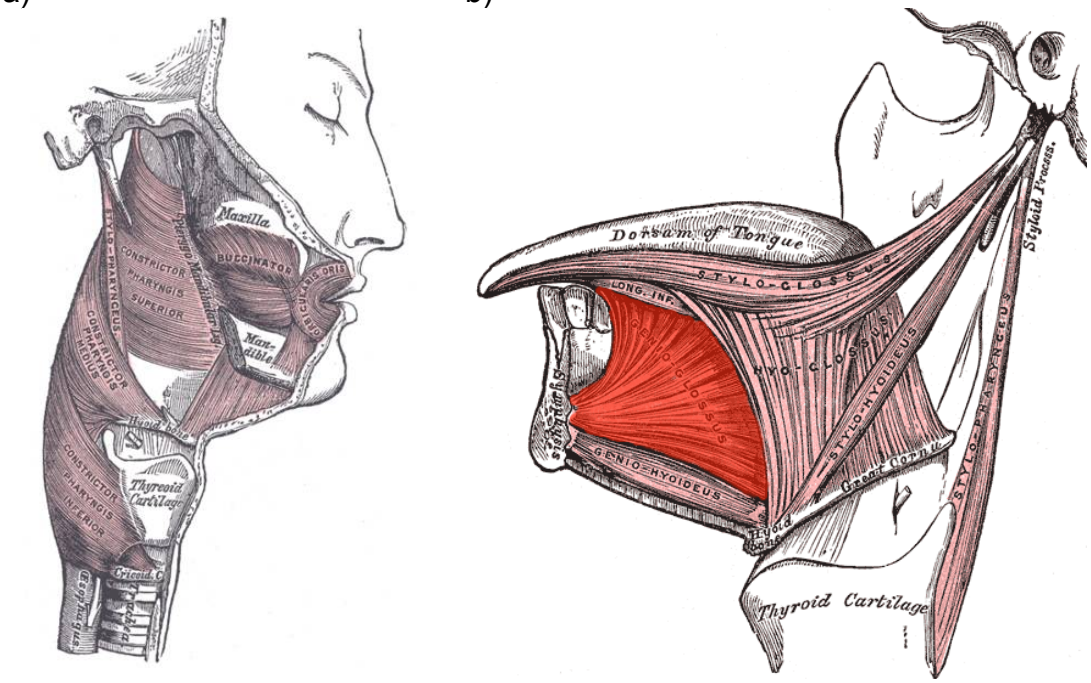


Figure 2-3 Pharyngeal muscles : a) Pharyngeal constrictor muscles: superior, middle and inferior [94], b) Pharyngeal dilator muscles in glossus: genioglossus and geniohyoid ([94] modified by Uwe Gille)

Pharyngeal dilator muscles

The upper airway dilator muscles are a loose collection of geographically unrelated muscles that counteract the forces of the pharyngeal constrictor muscles. The upper airway dilator muscles include the genioglossus, and geniohyoid shown in Figure 2-3 b, tensor veli, sternothyroid and sternohyoid in Figure 2-4 a and b [95].

During inspiration, outward dilating forces are generated by contraction of skeletal (or striated) pharyngeal dilator muscles surrounding the upper airway to prevent it being closed by the negative intrapharyngeal pressures [25, 92, 96-98]. The negative pressure reflex is the major inspiratory stimulus for pharyngeal stability of the upper airway dilator muscles [89, 98-103] and their activity increases proportionally with increased respiratory effort [93].

The airway response to negative pressure in the upper airway is not confined to airway dilator muscles but also involves increased drive to tongue retractor and pharyngeal constrictor muscles [104].

a) b)

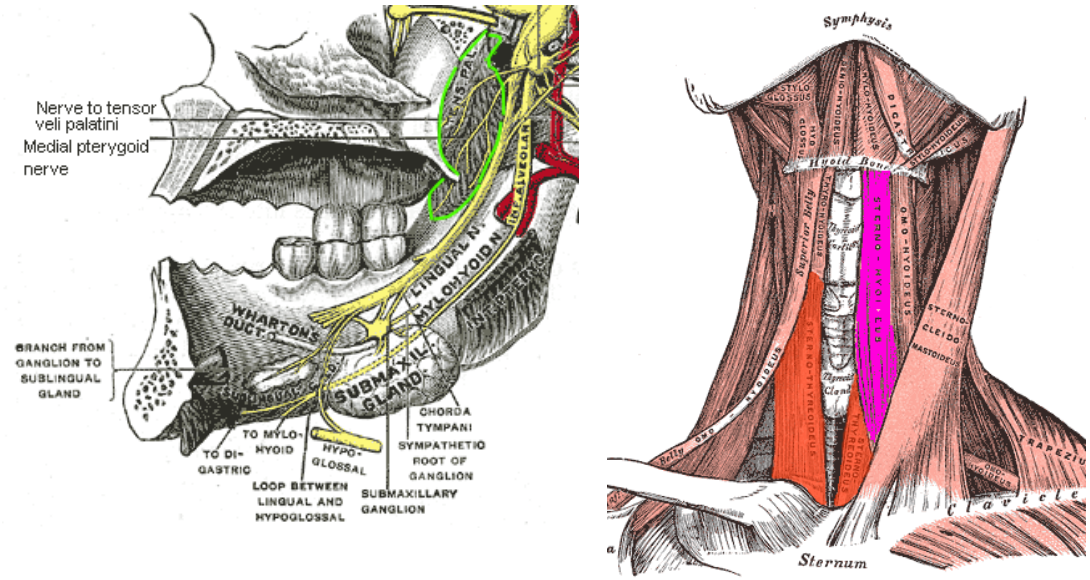


Figure 2-4 Pharyngeal dilator muscles : a) tensor veli palatine ([94] modified by author), b) sternohyoid and sternothyroid ([94] modified by Uwe Gille)

Inspiratory activity of the upper airway muscles slightly precedes that of the diaphragm, to prepare the extrathoracic airway for an inspiratory decrease in intraluminal pressure [92, 105]. During expiration the pharyngeal dilator muscles become less active [106]. The contraction has both central and reflex components [107].

Increases in lung volume causing longitudinal traction assist activation in keeping upper airway dilator muscles open [108].

The upper airway dilator muscles are not specifically designed for their respiratory role and may fail in the presence of increased loads, such as in obstructive sleep apnoea [93]. Only a few kilopascals of subatmospheric pressure in the pharynx tends to pull the tongue posteriorly and cause the pharynx to collapse [25].

Innervation of upper airway

While the main effect of the vagal nerve, X, is on parasympathetic control of internal organs and the heart, it also has motor control of the striated muscle of the pharyngeal constrictors and keeps the larynx open for breathing via action of the posterior cricoarytenoid muscle (Table 2-9, Table 2-10). The

glossopharyngeal nerve, IX, also innervates the pharyngeal constrictors [109, 110].

The pharyngeal dilator muscles are activated by two cranial nerves, V and XII (greyed cells in Table 2-11). The trigeminal nerve, V, is mainly sensory, but includes motor fibres that control biting, chewing and swallowing and controls one of the pharyngeal dilators, the tensor veli with mainly tonic activity [34]. The hypoglossal, XII, comes from the medulla oblongata, the lower half of the brainstem that contains cardiac and respiratory centres (and also vasomotor and vomiting). It controls the other pharyngeal dilators with phasic activity during inspiration [34].

2.2.5 Normal respiration

Respiratory cycle modulation

The upper airway (glossal, suprahyoid, infrahyoid, and pharyngeal) muscles all show EMG activity that increases during inspiration and returns to background level during expiration. There is synchronous activity of the majority of the pharyngeal dilator and constrictor muscles suggesting simultaneous contraction of these antagonistic muscles is needed to maintain pharyngeal airway patency [111].

The complexity of the airways makes it difficult to assign reflex activation of pharyngeal dilator muscles to specific receptors [106]. The way the pharyngeal muscles function in an integrated function to alter the size and shape of the pharyngeal airway is still being investigated but cross-muscle activation appears important [112].

Table 2-9 Innervation of soft palate and glossus with cranial nerves X and XI

			Nerve:	X Vagus	XI Accessory
			Sensory:	Velum Posterior and inferior pharynx Larynx	NA (Motor only)
			Motor:	Pharynx and larynx (speech and breathing)	Neck Cranial = vagus
Location	Muscle	Action	Increase activity	Parasymp organs neck to colon	
Soft palate (velum)	Musculus uvulae (sound and snoring)	Raise uvula, shorten velum, stop food in nose, oral breathing		X (pb)	XI [113]
	Levator veli palatine	Raise soft palate to stop food in nose, oral breathing		X (pb) [113]	XI [113]
	Palato-glossus 2 (also see Glossal)	Raise velum, tongue root up and back (nasal breathing)		X [113]	
	Palato-pharyngeus	Depress velum, constrict pharynx (nasal breathing)	INSP [111]	X	XI [113]
	Tensor veli palatine	Tense soft palate in mastication, move up & down			
Glossal (tongue extrinsic)	Palato-glossus 1	Depress soft palate		X (pb)	XI

Abbreviations: INSP, inspiratory; NA, not applicable; n/m, not modulated; pb, pharyngeal branch; Parasymp, parasympathetic.

Table 2-10 Innervation of pharynx and larynx with cranial nerves IX, X and XI

			Nerve:	IX Glosso	X Vagus	XI Accessory
			Sensory:	Posterior 1/3 tongue, velum and pharynx	Velum Posterior and inferior pharynx Larynx	- Motor only
			Motor:	Pharyngeal	Pharynx and larynx (speech and breathing)	Neck Cranial = vagus
			Increase activity	Parasymp 3x salivary glands (parotid)	Parasymp organs neck to colon	
Location	Muscle	Action				
Pharynx - external	Superior & middle constrictor	Move food, stripping	INSP [111]	IX	X	Symp and motor
- circular layer	Inferior constrictor	Move food, stripping	EXP [111]	IX	X	Symp and motor
- internal	Stylo-pharyngeus	Elevate larynx in swallow	INSP [111]	IX	-	
-longitudinal layer	Salpingo-pharyngeus		INSP [111]		X	
Larynx (voicebox)	Crico-arytenoid Thyro-arytenoid Transverse arytenoid				X (recurrent laryngeal)	
- collapsible	Cricothyroid		INSP [111]		X (superior laryngeal)	

Abbreviations: EXP, expiratory; INSP, inspiratory; Glosso, glossopharyngeal; n/m, not modulated; pb, pharyngeal branch; Parasymp, parasympathetic; Symp, sympathetic; V3, mandibular.

Table 2-11 Innervation of soft palate (velum), suprahyoid, infrahyoid and glossus with cranial nerves: V trigeminal, VII facial and XII hypoglossal.

			Nerve:	V	VII	XII
			Sensory:	Face, ant 2/3 tongue	Taste ant 2/3 tongue	Motor only
			Motor	Bite chew and swallow	Face, chew (lips, cheeks)	All extrinsic and intrinsic tongue
Location	Muscle Pharyngeal dilators	Action	Increase activity		Parasymp saliva and nose	
Soft palate (and Table 2-9)	Tensor veli palatine	Tense soft palate in mastication, move up & down	Tonic [114]	V3		
Suprahyoid	Stylohyoid	minor	INSP [111]		VII	
	Stylohyoid	major	n/m [111]		VII	
	Digastric		n/m [111]	ant V3	post VII	
	Mylohyoid	Elevate hyoid bone and floor of mouth to aid swallowing		V3		
	Geniohyoid	Elevate hyoid bone or depress mandible	[115]			XII,C1
Infrahyoid	Sternohyoid Sternothyroid		INSP [111]			XII,C1
	Thyrohyoid		INSP [111]			XII,C1
Glossal (tongue extrinsic)	Styloglossus	Draw tongue up and back to swallow food	INSP [111]			XII
	Hyoglossus	Draw side of tongue down				
	Genioglossus	Pull tongue forward, stick tongue out				
	Palatoglossus 1	Depress soft palate		See Table 2-9		

Abbreviations: ant, anterior; C1, cervical spinal nerve 1; INSP, inspiratory; n/m, not modulated; pb, pharyngeal branch; Parasymp, parasympathetic; post, posterior; Sy, sympathetic; V3, mandibular.

Respiratory pattern

Respiratory modulation of the motor neurons is generated in the cortex by the central respiratory pattern generator which receives information indirectly from pulmonary and airway vagal afferents [116]. Three major receptor systems feed peripheral information to central respiratory neurons in the process of maintaining homeostasis: the peripheral arterial chemoreceptors, skeletal muscle and joint receptors, and the receptors distributed within the lungs and airways [116].

Respiratory pattern varies because of transient metabolic changes, speech, postural changes, alterations in the state of consciousness (e.g. sleep, or anaesthesia), or effects related to pathological disturbances (e.g. Cheyne-Stokes) [117].

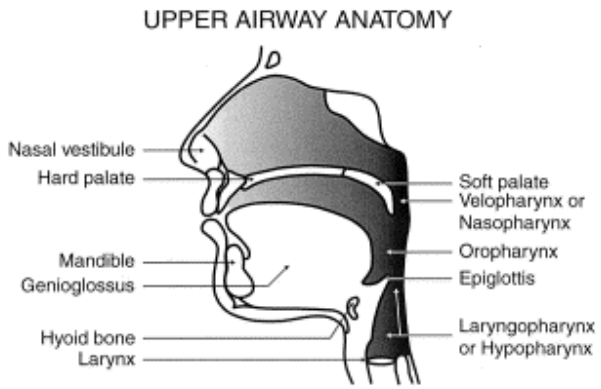
Volitional alterations in respiratory rhythm and drive in association with speech, singing, and other similar activities suggests a cortical centre capable of inhibiting the pattern generator and activating the muscles of respiration, including the diaphragm. Activation of cerebral cortical neurons can inhibit brainstem respiratory drive and directly activate spinal respiratory muscle motor neurons [118].

2.2.6 Respiratory pattern in sleep

Muscle tone throughout the body decreases during sleep including relaxation of the upper airway dilating muscles (normal action shown in Figure 2-5 middle). Suppression of motor output to the pharyngeal muscles during sleep promotes airway closure [119] resulting in considerable upper airway narrowing during inspiration, which leads to snoring [98], and in the extreme condition, apnoea [103, 117]. The airway collapse can occur in three places (Figure 2-5 c).

Loss of excitatory inputs to the hypoglossal motor neurons greatly decreases the ability of the genioglossus and other upper airway dilator muscles to respond to negative pressure and other stimuli that reliably activate these muscles during wakefulness [120].

a)



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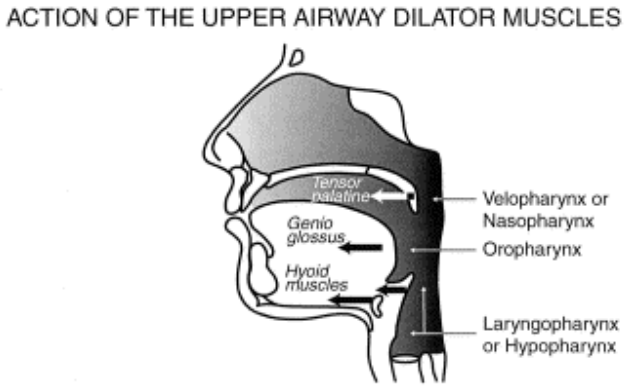

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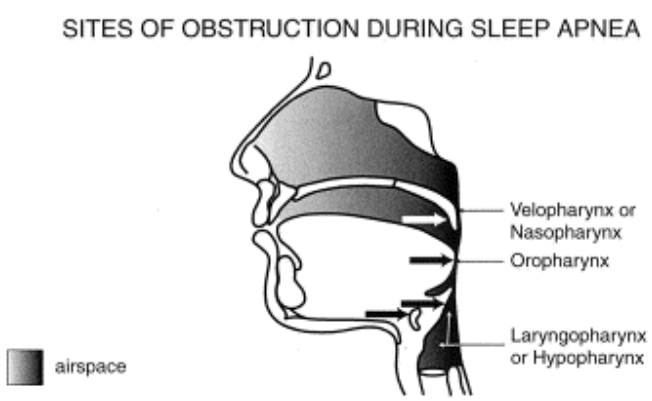


Figure 2-5 Upper airway anatomy, action of dilator muscles and sites of obstruction. Actions of the tensor palatine, genioglossus, and hyoid muscles enlarge the nasopharynx, oropharynx, and the laryngopharynx, respectively. Collapse is shown in 3 places: nasopharynx at the palatal level, the oropharynx at the glottic level and the laryngopharynx at the epiglottic level. Reprinted from [34] with permission from Elsevier.

When the airway collapses during sleep (obstructive sleep apnoea), ventilation is reduced (hypopnoea) or absent (apnoea) and hypoxia and hypercapnia may develop [114]. This reduction in ventilation causes changes to blood gas that increase respiratory drive until the upper airway re-opens (which is often associated with an arousal from sleep), at which time ventilation increases to reverse the blood gas abnormalities. Both the blood gas disturbance and arousal from sleep stimulate the sympathetic nervous system.

2.2.7 Opioid effects on respiration

The opioid effects of interest to this study are on respiratory drive and rhythm, and upper airway muscle tone.

Lalley [121] found threshold intravenous doses of fentanyl in the cat slowed discharge frequency and prolonged duration without affecting peak discharge intensity of respiratory neurons. The effects on three types of vagal motor neurons (vagal post-inspiratory - laryngeal adductor, vagal laryngeal abductor and pharyngeal constrictor) might explain tonic vocal fold closure and pharyngeal obstruction of airflow. Lalley found both inspiratory and expiratory bulbospinal neurons were clearly inhibited by mu-opiate agonists.

Opioid effect: respiratory drive and rhythm

Opioids that affect μ-receptors (e.g. morphine, fentanyl) cause depression of respiration by activation of the μ-receptor, primarily through a direct action on brainstem respiratory centres, though the details are unclear [6].

Fentanyl is widely known to induce significant central respiratory depression and increase cardiac vagal tone [75, 122, 123]. Respiratory depression induced by fentanyl and sufentanil has been measured by end-tidal CO_2 ($ETCO_2$) and ventilatory and occlusion pressure responses to CO_2 rebreathing [124].

Opioid effect: upper airway tone

All central depressant drugs diminish the action of the pharyngeal dilator muscles promoting pharyngeal collapse. The commonly used anaesthetic drugs that have been demonstrated to cause pharyngeal collapse include opioids [30, 80] and benzodiazepines [42, 125].

Mechanisms that lead to airway obstruction in obstructive sleep apnoea and during anaesthesia demonstrate physiologic similarities, including reduced tonic activity of the upper airway musculature, reduced lung volume, a considerable reduction in the critical pressure for airway closure, and failure of phasic activation of the upper airway musculature to precede diaphragmatic activity [92].

A combination of depression of central respiratory output to upper airway dilator muscles and of upper airway reflexes has been associated with a dose-related inhibition of genioglossus muscle activity during propofol anaesthesia [126]. More recently it has been determined that fentanyl decreases tongue muscle activity by suppression of the motor output (μ-receptor stimulation) from a central respiratory motoneuronal pool that activates the genioglossus muscle [127].

2.2.8 Summary opioid effect

The multiple tasks performed by the upper airway (breathing, swallowing, and speaking) make it a weak point in the airway structure. Normal respiration requires stiffening of the upper airway to prevent collapse during inspiration. This is accomplished with phasic vagal modulation of the pharyngeal dilators (and associated activity in the other airway muscles, particularly the pharyngeal constrictors). The vagal nerve, cranial nerve X, as well as being in control of the heart rate, controls the pharyngeal constrictors, larynx and soft palate.

The pharyngeal dilators are controlled by a combination of the V and XII cranial nerves: V, trigeminal, controls the tensor veli palatine used to move the soft palate up and down in mastication; and XII, hypoglossal, controls the other three pharyngeal dilators: geniohyoid, sternohyoid, and genioglossus, also heavily involved in eating swallowing and tongue control.

Opioids generally affect the autonomic nervous system decreasing sympathetic activity and increasing parasympathetic activity and vagal tone. This decreases activity in motor neurons driving respiratory drive and rhythm and decreases upper airway tone.

Inspiratory airway patency is modulated by autonomic nervous system respiratory centres; patency is reduced by increased vagal activity in pharyngeal constrictors and decreased activity in pharyngeal dilator motor neurons.

Chapter 3. Background: The heart and heart rate variability

We know that opioids depress respiration and also depress heart rate. Respiratory modulation and autonomic nervous system activity are reflected in changes to the heart rate that can be measured by heart rate variability (HRV).

3.1 Anatomy

The heart pumps blood through the lungs and around the body. Its activity is moderated by the autonomic nervous system depending on many factors, particularly a) the presence of oxygen in the lungs and b) the need for oxygen by the body. The requirement for oxygen varies from the minimum required for basal metabolism during sleep to the peak required for maximal exertion. The range of this requirement affects the rate of heart beat, the speed of contraction and the amount of time between beats.

3.2 Innervation

The autonomic nervous system controls the rate and amount of blood being pumped in each beat with two control lines: the sympathetic and parasympathetic nervous systems. The two systems act in opposition and can be likened to the accelerator and brake in a car. It is often described as a reciprocal relationship [128], but there is also selective action of either system; it is the resultant balance between these systems that sets the heart rate [129].

The sympathetic system enables the blood flow to be increased to meet external demands placed on the body – from the simple act of standing up, to responding to life threatening danger.

The parasympathetic system aims to conserve and restore energy; heart rate is reduced, contraction rate is slowed, and systems for eating and digestion are enhanced.

These two systems arise from different areas of the brain and predominantly act on different components of the heart.

3.2.1 Sympathetic

The sympathetic nerves arise from the cervical and upper thoracic portions of the sympathetic trunks. The fibres pass through the cardiac plexuses and terminate on the sinoatrial and atrioventricular nodes, on cardiac muscle fibres and on coronary arteries. The sympathetic nerve has its largest effect directly on the muscle of the ventricle, the main pump, with the ability to increase the force of contraction enabling a faster heart rate. Activation of the sympathetic system results in cardiac acceleration, increased force of contraction of the cardiac muscle, and dilatation of the coronary arteries [130].

3.2.2 Parasympathetic

Parasympathetic activity, via the vagus nerve, primarily controls heart rate at the sinoatrial node, the electrical pacemaker of the heart. Parasympathetic cardiac vagal neurons arise from the nucleus ambiguous of the upper medulla [131]. Activity of the cardiac vagal neurons is intrinsically silent; they do not display any pace-maker activity like brainstem neurons involved in maintaining blood pressure or respiration. The cardiac vagal neurons do not fire until they receive synaptic input [132], but they respond instantly even to small inputs [129, 131].

Usually ongoing tonic activity is present on cardiac vagal neurons; they are influenced by arterial baroreceptors and chemoreceptors, and are susceptible to pain, trauma or anaesthesia [131].

3.2.3 Latency

The two systems affect heart control differently through the sinoatrial node; the parasympathetic effect is almost instantaneous with the ability to change the rate each beat [133], while the sympathetic activity takes a few seconds (Figure 3-1) [134]. These differences might be explained by differences in nervous conduction times, width of synaptic cleft, kinetics of receptor activation and post-receptor events [135].

Vagal control of heart rate is through the release of acetylcholine. The effects are fast and short-lived and compared to the sympathetic effects of norepinephrine on the sinoatrial pacemaker frequency [135]. Early studies of

the pharmacological responses showed the interactions to be mutually inhibitory [136]. Later studies showed more complex non-additive interactions with accentuated antagonism: concurrent sympathetic activation exaggerating the effects of vagal stimulation on the heart [137]. The vagal system has the ability to override the sympathetic: high levels of vagal activity minimising the effect of sympathetic activity on heart rate [136].

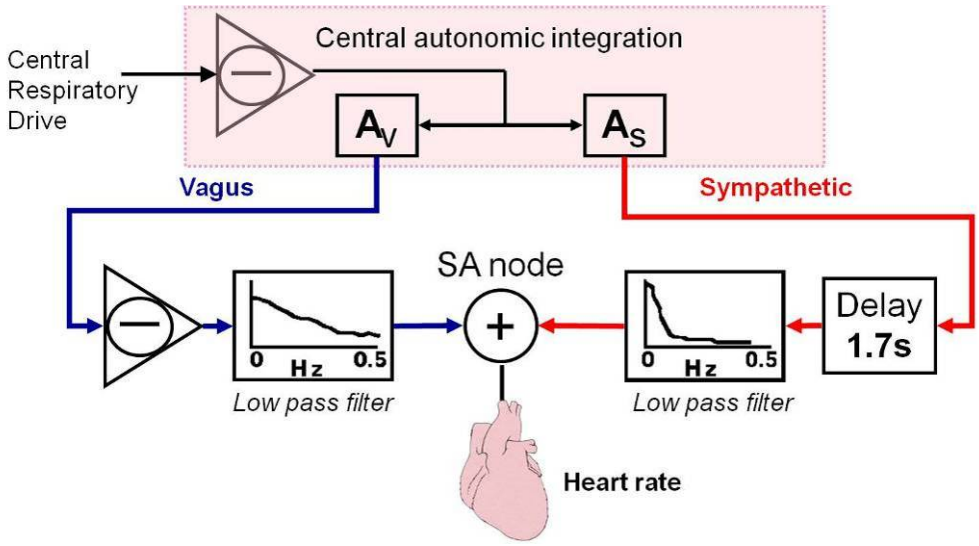


Figure 3-1 Model of heart rate control by sympathetic and parasympathetic effects on sinoatrial (SA) node (modified from [138])

3.2.4 Modulation

Heart rate via the autonomic nervous system is internally modulated by many other feedback mechanisms (Figure 3-2) including: respiration (further detailed in Figure 3-3), chemoreceptors (CO_2, O_2), baroreceptors (aortic pressure) and temperature [139-141]. The system of heart rate modulation is a complex interaction of many components [142].

3.2.5 Intrinsic heart rate

The sinoatrial node has a natural rhythm of about 43% above resting heart rate [143]. This intrinsic heart rate is the default when autonomic nervous system activity is removed (as in denervation or pharmacological blockade). At heart rates below the intrinsic rate, during normal daily activities and sleep, heart rate is regulated mainly by parasympathetic activity [144].

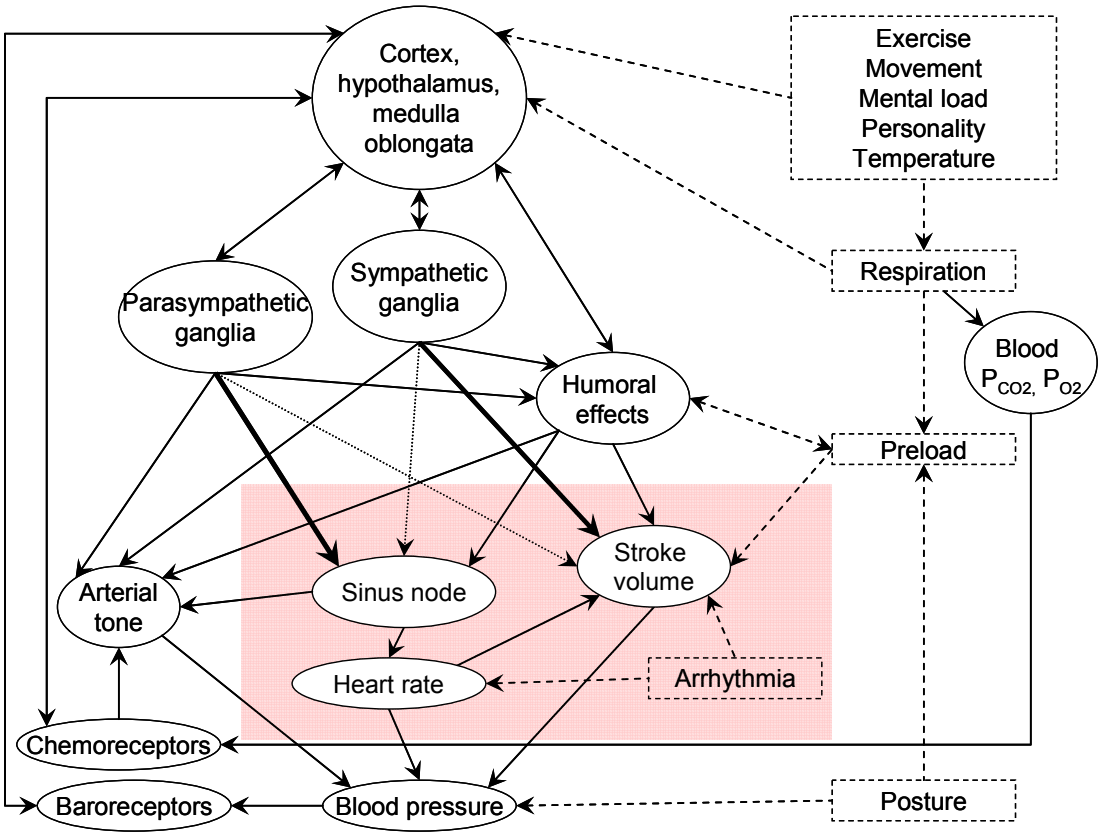


Figure 3-2 Simplistic model of heart rate regulation (adapted from [142]) Additional factors with considerable influence on HR are shown within the dashed boxes. Darker and lighter lines emphasize the difference in sympathetic and parasympathetic control of the heart (shaded box). Abbreviations: P_{CO2}, Arterial carbon dioxide tension; P_{O2}, arterial oxygen tension.

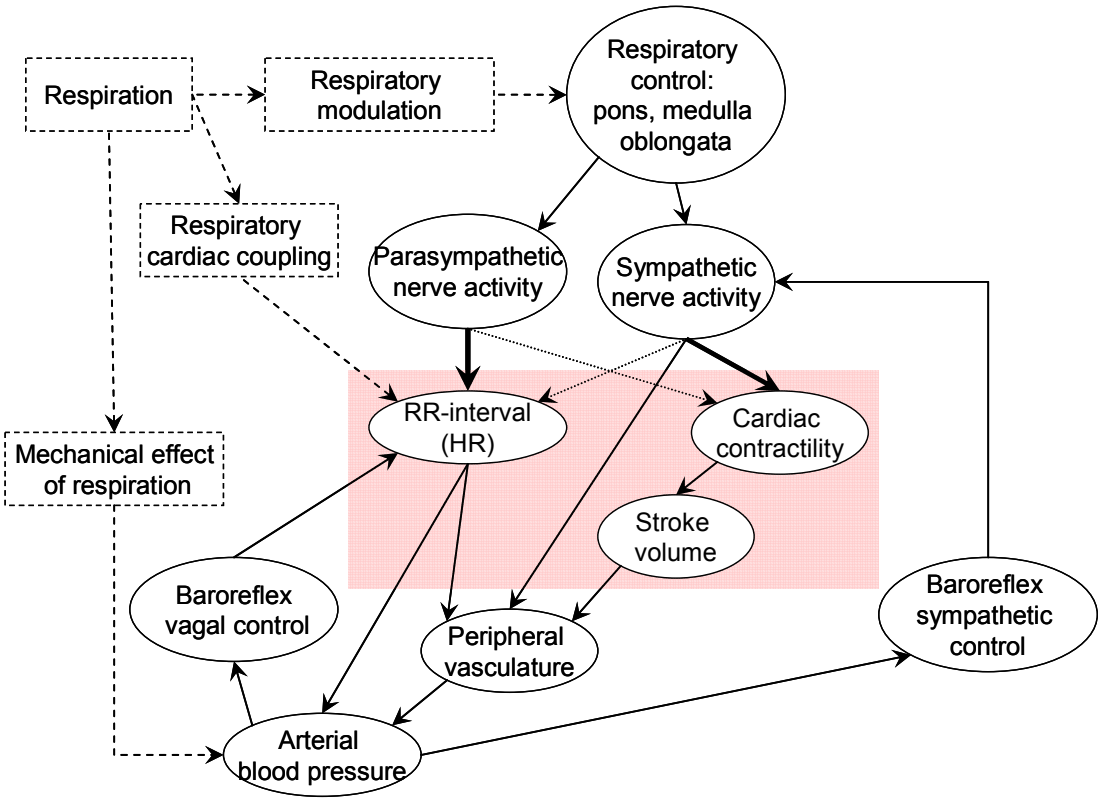


Figure 3-3 Diagram of the interrelationships among respiration and cardiovascular variables and how they contribute to RR-interval (adapted from [145])

3.3 Respiratory sinus arrhythmia

During respiration, heart rate increases during inspiration (inspiratory tachycardia) and decreases during post-inspiration and expiration. This respiration effect is termed respiratory sinus arrhythmia, RSA. The dominant source of RSA is not from pulmonary stretch receptors, respiratory-related changes in venous return, or cardiac stretch, but from the brainstem [146].

Katona and Jih [147] found that RSA is mediated by cardiac vagal activity with a reduction in cardiac vagal activity during inspiration; with artificial ventilation and no spontaneous respiratory activity, modulation of vagal activity disappeared. RSA is augmented by a drop in breathing frequency or an increase in tidal volume [148]. It exists when the lungs are not moving (e.g. when paralysed or with constant flow ventilation) and is reduced in high heart rate conditions of pain and anaesthesia [131].

The neurons and mechanisms within the central nervous system responsible for any type of cardiorespiratory interaction are still being determined [131]. The few data that exist suggest the cardiorespiratory interactions occur within the nucleus ambiguous. Three mechanisms may be responsible for the genesis of the RSA [148]: a) medullary respiratory neurons could regulate vagal cardiovascular motor neurons directly in synchrony with the respiratory cycle, b) blood pressure changes could indirectly modulate heart rate via arterial baroreceptors, and c) lung inflation could cause a reflex response mediated by lung stretch receptors.

It has been proposed that RSA improves the efficiency of pulmonary gas exchange by matching ventilation with pulmonary blood flow [149] but this theory is disputed [150, 151].

3.4 Heart rate variability, HRV

3.4.1 HRV basics

HRV is a measure of the variability of the heart rate and uses beat-to-beat changes in heart rate to determine activity of the sympathetic and parasympathetic nervous systems. The beat-to-beat measure is the time between the large, easily detectable R-waves of the ECG, RR-intervals (Figure 3-4). There are a variety of techniques for quantifying this variability. The standard techniques refer to traditional time domain and geometric methods, and to spectral analysis. Other methods include: respiratory sinus arrhythmia, Poincaré plots, heart rate characteristics and nonlinear methods. Following is an introduction to these techniques.

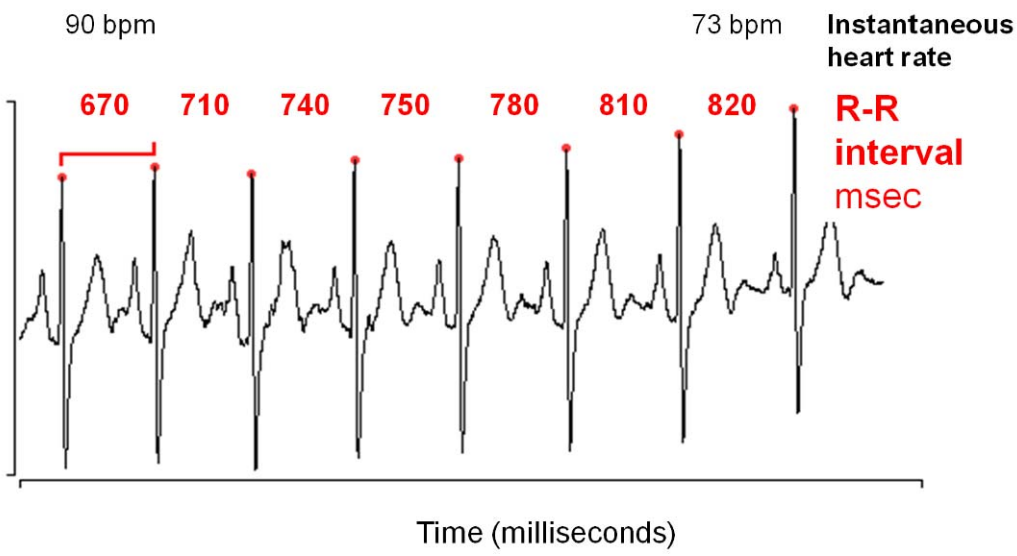


Figure 3-4 Beat to beat changes, RR-intervals, measured between the ECG R-waves

Time domain and geometric methods

The traditional indices use statistical methods to provide information on total variability and short-term vagal activity: SDNN, standard deviation of the normal sinus beats; RMSSD, root mean square of successive differences between beats; SDSD, standard deviation of successive differences; NN50 and pNN50, the count or proportion of intervals with differences of more than 50 ms. These indices require careful editing of the ECG to identify abnormal beats. Simple statistical measures of the RR-intervals and the interval differences have proven to be useful clinical tools [152]; depressed HRV can be used as a predictor of risk after acute myocardial infarction and as an early warning sign of diabetic neuropathy [152]. Geometric methods quantify the shape of a histogram of the intervals.

Spectral analysis HRV

Spectral analysis is useful in separating the effects of parasympathetic and sympathetic control. The 0.15 Hz low pass filter on sympathetic activity enables spectral analysis to partially separate the two systems [152].

The high-frequency component (HF, 0.15-0.4 Hz) is mediated by the parasympathetic nervous system, and the low-frequency component (LF, 0.04-0.15 Hz) is mediated by both the sympathetic and parasympathetic nervous

systems. The ratio of low-frequency to high-frequency (LF/HF) is considered to be a useful index of cardiac sympathetic nerve activity [153, 154].

Spectral analysis is commonly performed with fast Fourier transforms (FFT) but can also be done with autoregressive techniques, wavelet transforms, or the Lomb periodogram [155, 156].

The FFT requires pre-processing of the RR-interval time series to transform and filter the data: resampling to give equal time intervals (e.g. cubic spline interpolation), trend removal, and application of a window to decrease edge effects and spectral leakage (e.g. Hamming window) [152, 157].

Autoregression uses a set of autocorrelation functions to compare the signal to itself at different time lags providing information on whether the signal is periodic. Each value of the series can be predicted as a weighted sum of previous values based on the assumption that the most recent data points contain more information than the other data points [158]. It requires specification of the type of the model, the number of samples, the central frequency for each component and the value of the model order (number of parameters) [152]. The model must have goodness-of-fit calculated (e.g. residuals) in order to test the reliability of the model [155, 159]. The autoregressive approach is less sensitive to step changes and can be used with shorter time series (64-128 RR-intervals) that are more likely to be stationary [160, 161].

Wavelet transforms break a signal into various shifted and scaled versions of the original signal. The signal is repeatedly convolved with a low-pass and a high-pass filter and downsampled. It is able to analyse signals at multiple scales and is not affected by discontinuities [162].

Complex demodulation shows continuous changes in the amplitude of specific frequency components [163, 164].

The Lomb method is based on a least squares fit of sinusoids and does not require pre-processing of the FFT [165]. It performs the calculation directly on

unevenly spaced samples [166] avoiding the low-pass effect of resampling [165].

Spectral analysis with FFT has some drawbacks when used over short signal periods: it assumes the signal is stationary and it is recommended the recording lasts for at least 10 times the wavelength of the lower frequency bound of the investigated component, and in order to ensure the stability of the signal, should not be substantially extended [152]. A minimum recording time of ~1 min is needed for HF, 2 min for LF[2] and 5 min is preferable for standardization over different studies [152].

Standard time and spectral methods all have a close mutual correlation for long-term 24 hr analysis [152, 167].

Respiratory sinus arrhythmia, RSA

Respiration-related cardiac vagal changes where the heart rate increases with inspiration are measurable with HRV (Figure 3-5) [168, 169].

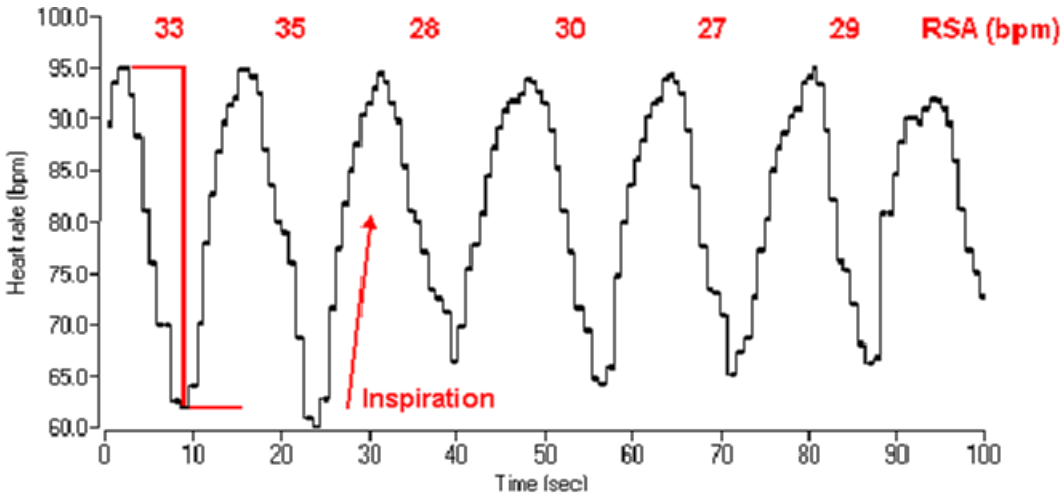


Figure 3-5 Measuring RSA from the tachogram: peak-valley method

The simplest measure of RSA is the difference between the fastest and slowest heart rate (peak-valley) within a breath [170] but this requires monitoring of the respiratory cycle. The peak valley method normally uses filtering at 0.1 to 0.4 Hz to remove non-respiratory frequencies. This filtering

[2] Note: The Task Force recommendations are confusing as the lower limit of LF at 0.04 Hz requires 250 s (i.e. 10/0.04) which is 4.17 min, twice as long as the 2 min recommendation. A 2 min record length would only capture a lower limit of 0.083 Hz.

may be detrimental if assessing slowing of normal respiratory rates. More complex is the Vagal Tone method patented by Porges [171] with a 3rd order, 21 point moving polynomial filter.

Other methods characterise the magnitude of the RSA with the mean or median of the absolute difference in heart period [172, 173].

The magnitude of RSA is equivalent to the HF component of spectral analysis [149], and also to RMSSD [174] for periods from 5 min to 1 hr. Changes in breathing pattern were not responsible for the decrease in the size of the HF component of HRV [75].

Poincaré plots

Poincaré plot is the common name used for a scatter plot to analyse heart rate variability (HRV) where the time between R-waves on an ECG, the RR-interval, is plotted against the succeeding RR-interval. A typical plot can be seen in (Figure 3-6). These plots, first used by Shaw in 1984 [175] to describe a dripping tap with random nonperiodic behaviour and chaotic activity, are also known in metrology as Lorenz plots [176], in mathematical areas as single return maps [177], and in physics as next amplitude plots [178]. They are able to reveal patterns resulting from nonlinear processes and nonperiodic fluctuations, however, the geometric measures of these plots all measure linear aspects [179].

Poincaré plot indices have advantages because the data has no requirement for normal distribution as with summary statistics, no requirements for: stationarity [180], minimum data set (*cf.* the low frequency spectral peak needs a two min recording [152]) or special processing that spectral analysis requires, and is more resistant to the influence of ectopic beats and other arrhythmias [177].

There are a variety of ways to characterise the scatter of points of the Poincaré plot: patterns, length and breadth, ratios, dispersion, area, asymmetry, and density.

Poincaré plots have been classified based on the pattern they display (comet, cigar, fan, torpedo, ball, and butterfly). Their major use has been to identify different forms of heart disease [181, 182] and stages of infant development [183].

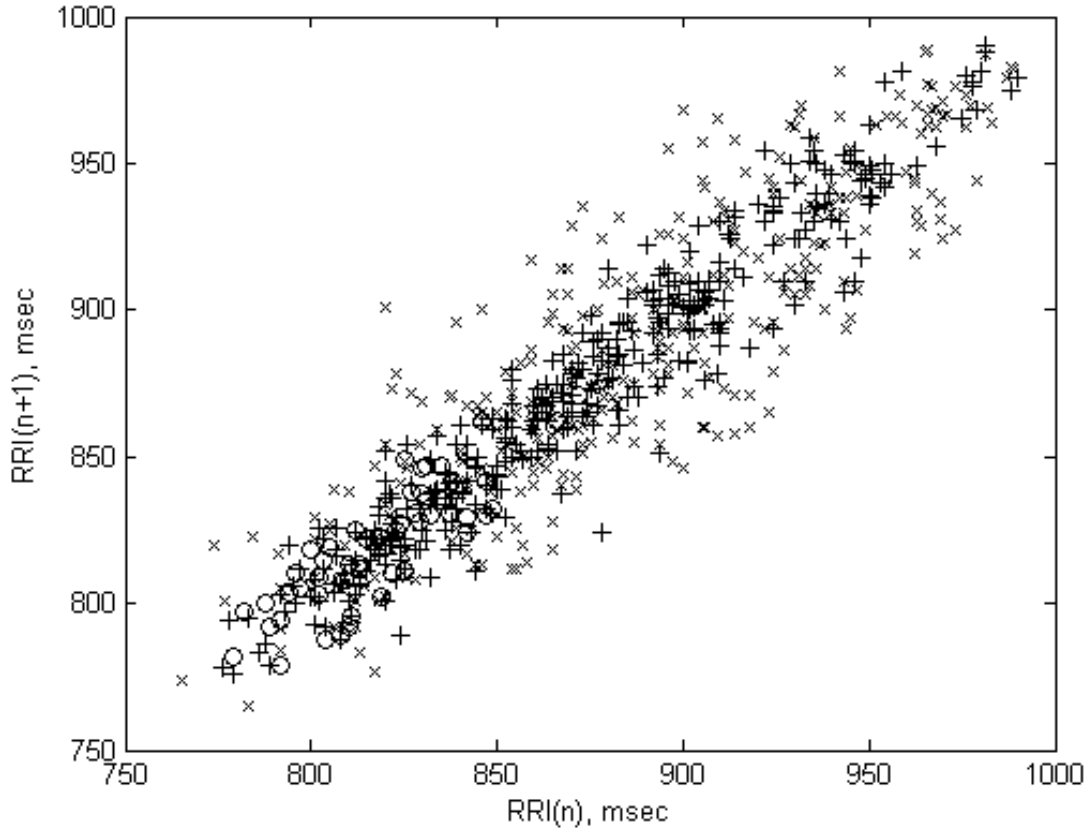


Figure 3-6 Poincaré plot for 10 min of RR-intervals x = baseline, o = fentanyl administration, + = post-fentanyl

Density and 3D indices were developed to extract information from data superimposition of data (e.g. 86000 beats over a 24 hr period compared to 350-500 beats over 5 min) [183-188].

Although the Poincaré plot is nonlinear, many of the Poincaré indices, longitudinal and transverse calculations of standard deviation, are linear and are able to be derived from traditional time domain indices [179]. The short-term variability, SD1, is equivalent to a scaled SDSD, and this can be subtracted from the total variability, SDNN, to give long-term variability [179].

Heart rate characteristics

Other heart rate characteristics, while not formal measures of HRV, can provide additional information on changes to the cardiac autonomic system and are applicable to short-term analysis: normality, skew, kurtosis, and gradient [186, 189].

Nonlinear analysis

Nonlinear analysis methods use chaos theory and fractal mathematics [167]. Instead of assessing HRV magnitude, they estimate correlation properties and complexity with only weak correlations between these measures and standard measures [167]. Some of these measure: regularity with Approximate Entropy [190] or Sample Entropy [144], chaotic behaviour with Lyapnov Exponents [191], fractal dimensions with 1/f spectral behaviour [192, 193], point correlation dimension [190] or dimensional complexity [194], and intermittency [195].

Measures of chaotic behaviour have been found to be reliable on samples as short as 500 beats [196].

3.4.2 HRV measurement

Determination

Postural and pharmacological interventions are commonly used as standards for testing the quality of HRV indices to report on sympathetic and parasympathetic activity. The HRV indices most widely used for these tests are the standard time and spectral domain indices.

To test the vagal system in isolation [197], propranolol can first be given to block beta-adrenergic receptors and sympathetic activity. In the supine position, the vagal activity is often at a maximum. If this is followed with successive doses of atropine then muscarinic receptors are also blocked reducing the vagal activity. This double autonomic blockade leaves the heart beating like a metronome at its intrinsic rate [198].

Parasympathetic blockade with anticholinergic drugs such as atropine [199] has been used to test correlation of vagal HRV indices (HF, RMSSD, PNN50)

[200], reduction in RSA [201], and to isolate increased sympathetic activity when combined with exercise [180].

Short time periods

Long term heart rate variability (HRV) analysis characterises overall neural cardiac control with a single number and relates that to chronic disease and mortality. Traditionally, HRV indices are calculated with data collected over 24 hr [178, 184-188]. The time and frequency domain indices are well described when collected over such time periods, and are extensively used as markers of autonomic system activity [152] predicting risk of cardiovascular disease [152].

Short-term analysis, typically over 5 to 20 min [177, 202-206] looks at relatively stable subsets of data in response to an intervention. Most interventions can be applied with an impulse or step function and assumptions of stationarity are made even though the physiological response may still be changing throughout the measurement period.

Instantaneous responses of HRV to interventions have been used to characterise the deterioration of the autonomic nervous system in diabetic autonomic neuropathy. Interventions include standing from supine, and the Valsalva manoeuvre (forceful breath into closed airway or resistance of 40 mmHg) [207, 208].

Many applications of HRV are now interested in characterising dynamic, short term changes (<1min) [209-211].

3.5 Effect of airway on HRV

Many studies have found links between HRV and respiration or respiratory depression but few studies have investigated the link between airways and HRV.

3.5.1 Effect of respiration on HRV

Rate of breathing affects HRV, most obviously the RSA reflects cardiac vagal activity [147]. RSA increases with tidal volume and time between breaths, plateauing with breaths taking 10 s or more [148]. Paced breathing at the

same rate as spontaneous breathing decreases LF and LF/HF in the supine position [212]. Decreasing the breathing rate from 12 br·min^{-1} to 8 br·min^{-1} increases LF-band and LF/HF ratio and decreases HF-band in subjects with metronome spontaneous breathing via a ventilator or in anaesthetised subjects [213].

Use of a mask [214] or respirator [215] will increase tidal volume and decrease respiratory frequency, and hence affect HRV. Continuous positive airway pressure also affects HRV; subjects with congestive heart failure with CPAP at higher levels (8 and 12 cmH_2O) have increased HRV given by SD2 (standard deviation of the Poincaré plot along the line of identity) [216].

Many HRV studies hold the breathing rate and tidal volume constant to control the effect of these variables [213, 217, 218].

3.5.2 Effect of respiratory depression on HRV

The secondary effects of respiratory depression also affect HRV: hypercapnia, hypoxia, anoxia, and asphyxia. Hypercapnia and hypoxia acting on the arterial chemoreceptors are strong stimulants to sympathetic activity [219], though opioids significantly reduce the hypercapnic and hypoxic effects on ventilatory drive [6].

Hypercapnia

Most studies found that hypercapnia caused increased HF, with mixed results for meanRR, LF and LF/HF [213, 220-222]. These studies had different breathing protocols (constant tidal volume at 0.2 Hz or 0.25 Hz, or spontaneous breathing) and lasted varying times (3 to 15 min).

Sasano *et al.* [220] investigated $ETCO_2$ of 30, 40 and 50 mmHg with constant tidal volume and breathing frequency (15 br·min^{-1}) and found after stabilisation (time not given) that hypercapnia increased HF (and hypocapnia decreased meanRR and HF with increased trend for LF/HF).

With paced spontaneous breathing (12 br·min^{-1}) and constant tidal volume, Poyhonen *et al.* [213] found an increase in $ETCO_2$ from 5% to 6% for a 10 min

period increased HF and LF. (Decreased $ETCO_2$ decreased HF with no change to LF/HF, LFnu, or HFnu).

After 15 min of spontaneous breathing hypercapnic normoxia (5% inhaled CO_2) Brown *et al.* [221] found increased tidal volume (from 5.5 to 15 $L \cdot min^{-1}$) increased HF, and reduced meanRR and LF/HF.

Tzeng *et al.* [222] looked particularly for a change in RSA with hypercapnia ($ETCO_2$ 50 mmHg) and found significant increases in tidal volume, HF, and RSA amplitude with a decrease in LF/HF and meanRR.

Hypoxia

Hypoxia for many minutes has been shown to change the HRV with a tendency to decrease mean heart rate, SDNN, RMSSD, LF and HF [222-224].

DeBeck *et al.* [223] found hypoxia with 10% O_2 for 7 min during spontaneous breathing decreased meanRR, RMSSD, SDNN, LF and HF with no change to HFnu and LF/HF. The HRV was calculated using the last 5 min of the record, (after 2 min stabilisation).

Vagal withdrawal was investigated by Buchheit *et al.* [224] under 6 min hypoxic conditions (11.5% O_2) at rest and they found decreased RMSSD and HF. The SpO_2 was reduced by 7.8% (range 3.2-10.8). They also showed increased heart rate and a trend to decreased LF with no change in SDNN, LFnu or HFnu.

Tzeng *et al.* [222] studied 15 min hypoxaemia with SpO_2 of 90% (in normocapnic conditions, $ETCO_2$ maintained at 40 mmHg) with spontaneous breathing. They found hypoxia decreased meanRR, but found no change in LF, HF, LF/HF or RSA amplitude (the difference between the maximum and minimum values on the RSA pattern waveform after it was aligned with respiration).

Anoxia

Goncalves *et al.* [225] determined that within a minute of anoxia in ventilated rats, all spectral powers significantly increased, as did LF/HF indicating increased activation of the sympathetic system. The animals were

anaesthetised with ketamine, an analgesic and sedative that, like fentanyl, binds to opioid μ-receptors, and is associated with respiratory depression (though to a much lesser extent than fentanyl [6]).

Asphyxia

Another study in rats by Boardman *et al.* [226] measured post-asphyxia with 1 min HRV and found heart rate decreased by only a small amount and SDNN increased by a larger amount with asphyxia severity.

3.5.3 Effect of airway on HRV

Vagal activity is known to promote bronchoconstriction of the airways [87, 227]; this effect is limited to the smooth muscle of the trachea and bronchi, below the level of the collapsible upper airway.

Pichon *et al.* [228] provoked the bronchial airway with methacholine (non-selective muscarinic receptor agonist in the parasympathetic nervous system) for confirming asthma. Responders (with bronchial hyperresponsiveness) gave an increased SDNN, SD1, HF, and HFnu and showed that bronchial provocation increased parasympathetic modulation of heart rate. Methacholine challenge was also used by Langdeau *et al.* [229] for showing that airway hyperresponsiveness in athletes was related to parasympathetic activity given by SDNN, but also due to other factors. Lewis *et al.* [227] reviewed studies of bronchial changes in asthma and parallel changes in heart rate to find many, but not all, had an apparent association with HRV that needed further investigation.

Although there are many studies showing an association between HRV and the vagally controlled smooth muscle of the bronchi, studies of an association between HRV and the somatically controlled skeletal muscles responsible for upper airway stability are rare.

There has been a study on the link between reflex control of the laryngeal adductor and cardiac vagal motoneurons. Stimulation of sensory receptors (upper airway negative pressure, pharyngoesophageal and peripheral arterial chemo- receptors) by Paton and Nolan [230] has shown coupling between post-inspiratory laryngeal resistance and bradycardia. The laryngeal adductors

are below the pharyngeal constrictor muscles but this study supports the possibility of a link between upper airway stability and cardiac vagal function.

3.6 Opioid effect on HRV

3.6.1 Direct effect of opioids on the heart

The primary mechanism of opioids is to cause bradycardia through central neurally mediated mechanisms [6].

Although opioids can interact directly with cardiac myocytes and morphine can have a direct effect slowing the sinoatrial node and atrioventricular conduction, the direct effect on the heart tissue is minimal [6]. Opioids have been reported to have positive inotropy, increasing the rate and force of contractility, but morphine has also been reported to have no effect, and fentanyl to have little or no effect [6].

3.6.2 Effect of opioids with anaesthetic agents on HRV

HRV has been widely used to characterise the onset and effects of anaesthesia on the autonomic nervous system. Studies have used a package of different anaesthetic agents that often include an opioid (Table 3-1), and analyse the data before and after induction of anaesthesia. The induction usually includes intubation and mechanical ventilation though this is not always described.

Table 3-1 HRV studies of anaesthetic agents with opioids
Part 1: Anaesthetic agents used

First author and year	Anaesthetic agents
Johnson 1980 [231] Foetal HRV N = 1	Premed iv: droperidol 2.5 mg, F 0.05 mg, diazepam 5mg Ind: thio 250 mg, panc 7mg Vent: mechanical, N_2O Anae: F 200 µg in divided doses over 20 min
Latson 1990 [232] 3 min	Ind: suF 0.5 $\mu g \cdot kg^{-1}$ IV, Maint: suF 0.5 $\mu g \cdot kg^{-1} \cdot min^{-1}$ (total dose 3.0 ±0.8 $\mu g \cdot kg^{-1}$) Rlx: vecu 10 mg IV Vent: mask with 100% O_2
Estafanous 1992 [233] Post-intub vs. pre-anae	Premed: morphine 90 $\mu g \cdot kg^{-1}$, scopolamine 4 $\mu g \cdot kg^{-1}$ Anae: suF 5-8 $\mu g \cdot kg^{-1}$, vecu 0.12-0.15 $mg \cdot kg^{-1}$ in 3 min Vent: intubation 100% O_2 at 12 $br \cdot min^{-1}$

First author and year	Anaesthetic agents
Halliwill 1992 [234]	1) pentobarbital sodium 25 mg·kg^{-1} iv 2) thio 25 mg·kg^{-1} iv 3) halothane 2% 4) morphine 1 mg·kg^{-1}, a-chloralose 50 mg·kg^{-1} iv and urethan 500 mg·kg^{-1} iv
Pomfrett 1993 [235]	Ind: propofol 2.5 mg·kg^{-1} bolus Maint: N_2O, F 3 µg·kg^{-1}, propofol 10-8 mg·kg^{-1}·h^{-1} cont Rlx: vecu 0.1 mg·kg^{-1}
Deutschman 1994 [236] Peri-anae changes 2 min F only for intub	Premed: lidocaine 50 mg iv and O_2 Ind: propofol 2.5 mg·kg^{-1} iv Maint: propofol 150 µg·kg^{-1}·min^{-1} Vent: positive pressure mask >12 br·min^{-1} 5 min then Rlx: succ 1 mg·kg^{-1} or vecu 0.1 mg·kg^{-1} and F 100 µg or alF 100 µg iv 2 min then intubation: pos pres vent >12 br·min^{-1}
Pomfrett 1994 [237]	Ind: propofol 2.5 mg·kg^{-1}, F 100 ug Maint: N_2O and isof 0.85% or 1.7% = 0.65 and 1.2 MAC Rlx: vecu 0.1 mg·kg^{-1} Vent: mechanically via ET tube
Licker 1995 [160] Tracheal intubation with epidural vs. general anae	Premed: midazolam 7.5 mg Epidural: 1% lidocaine 6 ml Ind: F 2 µg.kg^{-1} iv, thio to loss reflex, vecu 0.1 mg·kg^{-1} 5 min then intub Vent: 18 br·min^{-1}
Zickmann 1996 [238] Pre-ind, post-intub and 15 min post-intub 5 min	Premed: flunitrazepam 2 mg, morphine 30 mg A--F 7.5 µg·kg^{-1}, midazolam, 0.075 mg·kg^{-1} B--F 12.5 µg·kg^{-1}, midazolam, 0.125 mg·kg^{-1} C--F 20.0 µg·kg^{-1}, midazolam, 0.200 mg·kg^{-1} D--F 7 5 µg·kg^{-1}, midazolam, 0.075 mg·kg^{-1} (bolus) Rlx: panc 0.1 mg·kg^{-1} Vent: assisted then controlled at 11 br·min^{-1}
Plazak 1999 [239] After premed, peri-intubation (naso or oro) and post-intubation 5 min	Premed: pethidine 75-100 mg, promethasine 25-50 mg, 100%O_2 Ind: atropine 0.5 mg, F 0.1 mg, panc 1 mg, thio 5 mg·kg^{-1}, suxamethonium 1 mg·kg^{-1} Post-intub: F 0.2 mg, panc 4-5 mg Maint: O_2 2 L·min^{-1}, N_2O 3 L·min^{-1}, F 0.1 mg every 20 min Rlx: panc 1 mg
Storella 1999 [240] After premed, peri-anae, day 1 post-op 15 min	Premed: included scopolamine Ind: F 40 µg·kg^{-1}

Abbreviations: alF, alfentanil; anae, anaesthetic; F, fentanyl; Ind, induction; intub, intubation; isof, isoflurane; iv, intravenous; maint, maintenance; MAC, minimum alveolar concentration; N_2O, nitrous oxide in oxygen; panc, pancuronium; premed, premedication; rlx, relaxant; succ,

succinylcholine; suf, sufentanil; supp, supplementary; thio, thiopental sodium; vecu, vecuronium.

Spectral analysis is the main method used when investigating the effect of anaesthetics on HRV with some studies using time domain indices, RSA, and nonlinear techniques (see Table 3-2). In general there is a decrease of heart rate, total autonomic nervous system activity, and LF/HF with a shift of the balance between parasympathetic and sympathetic activities, reducing or eliminating parasympathetic cardiac activity.

Table 3-2 HRV studies of anaesthetic agents with opioids
Part 2: HRV effects, post-intubation

First author and year	HR	Total power	LF	HF	LF/ HF	Other HRV measures
Johnson 1980 [231]						Visual range = D
Latson 1990 [232]			D	D		
Estafanous 1992 [233]				NC	D	
Halliwill 1992 [234]	D					For morphine SDNN = I RSA_{Vtone} = I
Pomfrett 1993 [235]						RSA_{circ} = D compared to recovery (not pre)
Deutschman 1994 [236]	D	D	D	D	D	
Pomfrett 1994 [237]						RSA_{circ} = D compared to recovery (not pre)
Licker 1995 [160]	D	D	D	D	NC	
Zickmann 1996 [238]	NC		D	D_{NS}	D	For groups B,C,D (not A) CV = NC RMSSD = D_{NS}
Plazak 1999 [239]	I		LFnu D	HFnu D	I_{NS}	
Storella 1999 [240]	NC					SDNN = D ApEn = D PD2 = D

Abbreviations: ApEn,approximate entropy; CV, coefficient of variance; D, decrease; HF, high frequency spectral power; HR, heart rate; I, increase; LF, low frequency spectral power; NC, no change; NS, not significant; nu, normalised units; PD2, point correlation dimension; RMSSD, root mean square of successive differences in RR-interval; $RSA_{Vtone,}$ RSA_{circ}, respiratory sinus arrythmia measured with Vagal tone or circular statistical analysis; SDNN, standard deviation of NN- or RR-intervals.

Heart rate effects

Heart rate does not always decrease. Muscle relaxants like pancuronium attenuate opioid-induced bradycardia [241]; their vagolytic properties overcome the vagomimetic[3] properties of opioids and this has been put forward as the main reason for an unchanged vagal tone with this combination [238].

Intubation effects

Many anaesthetic studies measure HRV during or after intubation or with mechanical ventilation. Intubation has been reported to stimulate sympathetic activity with increased LF/HF and heart rate though others have reported confusing results with time domain indices, SDNN and RMSSD [242], and others a drop in LF after intubation [243].

Respiration-related effects

Anaesthesia has been shown to decrease tidal volume, which could, in turn, alter RSA. Eckberg [244] reported that a 50% increase in tidal volume led to a 15% increase in peak-to-valley RSA.

Decreases in RSA have been related to depth of anaesthesia [168, 169, 245, 246]. The high frequency (HF) spectral index is one measure used to track cardiac vagal activity, however, under anaesthetic, the respiratory rate shifts power from this to the low frequency thus reducing the effectiveness of spectral indices to monitor sympathovagal modulation [247].

Ventilation at <8 br·min^{-1} is known to interfere with HF spectral peak so many studies use >10 br·min^{-1} [236, 238] or baseline timed breathing at the ventilation rate [75] to minimise this effect.

Effect of face mask

Few studies have compared use of an oxygen mask to normal breathing without a mask. Furutani *et al.* [248] applied a mask (without O_2) to subjects supine, and sitting on a bike ergometer. They found that LF/HF increased from supine rest to sitting, then decreased back to the level during supine rest with

[3] Vagomimetic is causing a decrease in heart rate

the face mask. The value of HFnu had the opposite effect: it decreased from supine rest to sitting, and then returned to that during supine rest when sitting with the face mask.

Effect of facemask and oxygen

Bartels *et al.* [219] comparison of facemask with air to facemask with O_2 showed a decrease in heart rate and further drop in LF/HF with supplemental 31% O_2 compared to compressed air alone for patients with chronic obstructive pulmonary disease.

Shibata *et al.* [249] found that mask and O_2 compared to mask and air at 15 br·min^{-1} increased RR-intervals, LF and HF with increasing functional inspired O_2 concentrations (10 min each at 21, 40, 70 and 100%) with no significant change in SDNN of LF/HF (from 0.51 to 0.65).

3.6.3 Effect of fentanyl on HRV

Fentanyl has been one of the opioid anaesthetic agents used in many HRV studies to monitor changes in the autonomic nervous system: during induction of anaesthesia [250-253], with surgical stimulation [243, 254], during labour [255, 256], comparing route of administration [257], and during post-operative recovery [240].

Fentanyl slows AV node conduction, and prolongs the RR-interval, the AV node refractory period and Purkinje fibre action potential duration [258].

Early studies on dogs in the 1970's [122, 123] showed fentanyl gave a dose-related depression of heart rate during halothane or chloralose anaesthesia with the majority of the action of fentanyl via vagal efferent impulses after the nucleus of the solitary tract, NTS. After bilateral cervical vagotomies the decrease caused by fentanyl was at most 10% of the decrease in innervated dogs [122]. More recent work has shown that fentanyl inhibits GABAergic[4] transmission to cardiac vagal neurons in the nucleus ambiguus, providing one mechanism for the opiate-induced bradycardia [132].

[4] GABA is gamma-aminobutyric acid, a neurotransmitter in the central nervous system

Fentanyl was first analysed with HRV in a case study in 1980 [231] looking at the effect of maternal fentanyl anaesthesia on foetal HRV with no change in maternal or foetal HRV during pre-medication, intubation, or inhalation of nitrous oxide. Post-intubation, fentanyl (200 μg in divided doses) was found to give a loss of foetal HRV (from 20 to <5 beat·min^{-1}).

3.6.4 Pre-intubation effect of fentanyl on HRV

Fentanyl is usually given as part of the anaesthetic package; consequently few studies have looked at the effect of fentanyl on HRV in isolation from other anaesthetic agents.

A selection of studies are given in Table 3-3 where fentanyl is the independent variable or where HRV results are provided before fentanyl administration and after fentanyl administration but before intubation. The general finding (

Table 3-4) is that fentanyl decreases total, LF and HF power [252] with either no change in heart rate (HR) [243, 251] or a decrease [250, 253], with the assumption that the effect of fentanyl is consistent across subjects.

Galletly *et al.* [250] found fentanyl decreased HRV in all frequency bands and saw a shift in power to <0.15 Hz but there was no major difference in HRV spectral response. They theorised fentanyl should exert a parasympathomimetic effect on HRV but found no greater increase in HF (absolute or proportional) power or decrease in LF/HF ratio in the fentanyl groups as one might expect if there was a change in the balance of autonomic tone towards parasympathetic predominance. They proposed that when studying patients who were breathing spontaneously it is probable that any respiratory depressant effect of the opioids might have offset the expected increase in HF power, as ventilatory drive correlates with HF power [259, 260] and any carbon dioxide retention caused by the opioids would have altered the balance towards increased sympathetic tone.

Table 3-3 HRV studies with fentanyl
Part 1: Anaesthetic agents used

First author and year	Study	Anaesthetic agents
Komatsu 1992 [251] Mechanical ventilation	Ind vs. intub N=7	Premed: morphine 0.1 mg·kg^{-1} Ind: F 50-70 µg·kg^{-1}, diazepam 0.21 mg·kg^{-1}, panc 0.15 mg·kg^{-1}
Latson 1992 [252]	Pre vs. post ind to LOC N=10	Premed 45 min: midazolam 3.5 mg + 2.8 mg Ind: suF 2.9 µg·kg^{-1}
Latson 1993 [254] Ventilation not stated	Surgical stimulation propof vs. isof intubated	No premed Ind: F 100-300 µg +vecu a) +thio + isof N=13 b) +propofol N=13 Supplementary F < 300 µg
Galletly 1994 [250] Spontaneous breathing with manual ventilation when needed	Pre vs. post ind Same effect with or with no F	Ind: F + propofol (half) or just propofol Isof or halothane + N_2O, half get F 1 µg·kg^{-1}
Storella 1995 [190] Ventilation not stated	Pre vs. post induction	Premed: morphine 0.1 mg·kg^{-1}, scop 6 µg·kg^{-1} Ind: F 50-60 µg·kg^{-1} Rlx: panc 0.1 mg·kg^{-1} for ET intubation
Kohno 1997 [75] Mechanical ventilation	Matched ventilation 5 min	15 br·min^{-1} with timed auditory signal Ind: F 10 µg·kg^{-1} Maint: continuous 15 µg·kg^{-1}·h^{-1} Rlx: vecu 0.2 mg·kg^{-1} then ET intubation Vent: 15 br·min^{-1}
Schubert 1997 [243] Ventilation 12 br.min^{-1}	Surgical stimulation vs. pre-ind vs. pre-intub	Premed intravenous: 0-3 mg midazolam Ind: thio, F 100-400 µg Post-ind ventilation 12-15 br·min^{-1} Succ or vecu or panc for ET intubation Maint: N_2O and isof or enfl
Riznyk 2005 [253] Facemask with 100% O_2 at 12 br.min^{-1}	Pre-ind vs. post-F vs. post-ind	Premed 1 hr: midazolam 7.5 mg Ind: F 3 µg·kg^{-1}, facemask with 100% O_2 After 5 min: thio or propofol until LOC Rlx after 5 min: rocuronium for ET intubation
Vettorello 2008 [247]	F and spontaneous or paced breathing	F 1 µg·kg^{-1} not enough for respiratory depression

Abbreviations: enfl, enflurane; F, fentanyl; Ind, induction; intub, intubation; isof, isoflurane; LOC, loss of consciousness; maint, maintenance; N_2O, nitrous oxide in oxygen; panc, pancuronium; premed, premedication; rlx, relaxant; scop, scopolamine; succ, succinylcholine; thio, thiopental sodium; vecu, vecuronium.

Table 3-4 HRV studies with fentanyl
Part 2: HRV effects, pre-intubation

First author and year	HR	Total power	LF	HF	LF/ HF	Comment on method and PSD
Komatsu 1992 [251]	NC	D	D	D	D	FFT 256 s
Latson 1992 [252]		D	D	D I HFnu		15 s overlap FFT 64 s post-ind D 60-225 s (p<0.01)
Latson 1993 [254]	NC NC	D D	NC LFnu D LFnu	NC HFnu I HFnu		
Galletly 1994 [250]	D	D*	D*	D* I HFnu		FFT 5-10 min (LF=0.02-0.08, MF=0.08-0,15)
Storella 1995 [190]	NC	D				700 beats, 10-15 min NC SDNN D ApEn, peak ApEn, avg PD2
Kohno 1997 [75]	NC		NC	D		Matched respiration 15 br·min^{-1}
Schubert 1997 [243] post-ind	NC	D	D	D	I	FFT 128 s repeated 32 s
Riznyk 2005 [253]	D	D	D NC LFnu	NC HF NC HFnu	NC	FFT 64 s repeat 15 s for 5 min
Vettorello 2008 [247]	D	NC	D LFnu	NC HFnu	D	AR 300 beat

* same change for mixed anaes with no fentanyl
Abbreviations: ApEn, Approximate entropy; AR, autoregressive power spectral density; avg, average; D, decrease; FFT, fast fourier transform; HF, high frequency PSD; I, increase; ind, induction; LF, low frequency PSD; MF, medium frequency PSD; NC, no change; nu, normalised units; PSD, power spectral density.

3.6.5 Respiration-related fentanyl effects

One of the earliest studies (1964) with morphine-chloralose anaesthesia showed a conspicuous sinus arrhythmia in dogs; the heart rate increased during the inspiratory movement of the animal, and, at the same time, the activity of cardio-inhibitory (Type I) fibres in the vagus nerve was either completely absent or decreased. Heart rate and fibre activity variations were always associated with the inspiratory efforts of the animal [261].

Many of the studies in

Table 3-4 make no comment on the effect of O_2 facemask or ventilation. It is known that a mask [214] or respirator [215] will increase tidal volume and decrease respiratory frequency. Studies comparing mask and air to mask and O_2 found it was the O_2 that caused increased vagal activity [219, 249, 262, 263].

Kohno *et al.* [75] minimised the effect of mechanical ventilation by using timed breaths at 15 br·min^{-1} before fentanyl and they ignored the intubation process. They found the HF component of HRV was significantly reduced by fentanyl anaesthesia during mechanical ventilation, while mean heart rate and LF did not change.

The use of timed breathing does have problems. It overrides the intrinsic control of respiratory activity while removing respiratory variability and also changes the bi-directional cardiac–respiratory interactions, therefore having other possible unintentional effects [264].

In the one study where fentanyl was given in isolation from other drugs to determine the effect of spontaneous or paced (20 br·min^{-1}) breathing, Vettorello *et al.* [247] found fentanyl caused sympathetic decrease and a trend to vagal activation with reduced heart rate, LFnu and LF/HF during both spontaneous and paced breathing however paced breathing by itself decreased total power and SDNN.

With no drug intervention, Patwardhan *et al.* [265] found a large decrease in RSA (HF) magnitude was associated with controlled breathing at the subjects spontaneous rate when all other parameters (breath-to-breath variation, respiratory frequency, tidal volume and inspiratory-to-expiratory ratio) were kept the same implicating mental effort as a cause for the change.

3.6.6 Summary of opioid effects on HRV

Studies of the fentanyl effects have used spectral HRV indices to characterise autonomic nervous system changes in response to this opioid. Using periods of 1 to 15 min, fentanyl is generally shown to decrease total and LF power. The effects on HF, HFnu and LF/HF power are variable.

When opioids are part of an anaesthetic package, the results are less consistent with decreases generally seen in LF and total spectral power. The use of mechanical ventilation or O_2 facemask affects the results.

Chapter 4. Selection of very short-term indices

4.1 Aim

This study surveys indices and identifies those that may be useful in very short-term analysis of autonomic nervous system activity.

HRV has previously been used to detect fentanyl-induced changes in the autonomic nervous system over 5-15 min periods. To monitor the dynamic changes in this study, HRV analysis over shorter periods (< 60 s) is needed. The peak onset of respiratory depression with fentanyl is 5-10 min. The effect of fentanyl is expected to change throughout the short action period without reaching a steady-state condition.

Later chapters will investigate minimum window size, normality and stationarity of data in short windows, correlations with common indices, and usefulness of indices with real data.

4.2 Background

4.2.1 Short-term HRV

Long term heart rate variability (HRV) analysis characterises overall neural cardiac control by a single value and relates that to chronic disease and mortality. Traditionally, HRV indices are calculated with data collected over 24 hr [178, 184-188]. The time and frequency domain indices are well described when collected over such time periods, and are extensively used as markers of autonomic system activity [152].

Short-term analysis, typically over 5 to 20 min [177, 202-206] looks at relatively stable subsets of data in response to an intervention. Most interventions can be applied with an impulse or step function and assumptions of stationarity are made even though the physiological response may still be changing throughout the measurement period.

More recently, analysis for periods <2 min have been used [226, 266] that still requires stationary data and a minimum of 100-128 beats for spectral analysis.

Very short-term HRV provides the possibility of investigating in detail the instantaneous effects of the autonomic nervous system on the heart, and tracking dynamic changes in real-time. The interest in this thesis is monitoring the dynamic physiological responses to the opioid fentanyl, a common pre-operative sedative that has a peak effect in 5 min [72] and has mixed effects on both sympathetic and parasympathetic activities [6]. Before short-term assessment of dynamic activities can be undertaken in this situation, HRV indices suitable for a small sample size need to be identified.

4.3 Method

4.3.1 Survey of HRV indices

A survey was conducted of published methods of HRV analysis from the 1990s to the present day looking for indices that could be applicable to very short time HRV analysis (<60 s) of fentanyl effects.

Literature was identified through searches of PubMed (U.S. National Library of Medicine, Bethesda, MD, USA). Search terms included combinations of the following keywords: heart rate variability, traditional, frequency domain, respiratory sinus arrhythmia (RSA), Poincaré plots, and short-term. References in these papers were examined for further relevant literature.

The HRV indices included in the keywords are known to have strong links to the autonomic nervous system and could be expected to change with fentanyl. Nonlinear indices were not specifically included in the survey as changes are not related to the autonomic nervous system and are usually calculated over long periods (300-8000 beats) [142, 196, 267, 268]; however, if nonlinear indices were found and were applicable to very short time analysis, they were included.

4.3.2 Coding and testing of indices

Where it existed, published coding was used for the index calculations in MatLab (The MathWorks Inc., Natick, MA, USA). The rest of the coding was developed by the author. In conjunction with coding, a series of test data was developed to check the indices.

4.4 Results: Survey of Indices

The survey of published methods of HRV analysis found 67 indices applicable for very short-term HRV analysis (Appendix A). Some of these indices have been identified in previous publications [269, 270].

4.4.1 Coding and testing of indices

The MatLab code for calculation of the indices and for generation of the test data to check the indices is provided in Appendix A.

The test data (Figure 4-1) was designed to test aspects of each index over any selected window size up to 49 beats. Some test data generated a single pattern; others generated repeating patterns and a few included generation of a signal using random seeds. During the coding process, mathematically equivalent indices (but with different names) were identified with selection of the more common index.

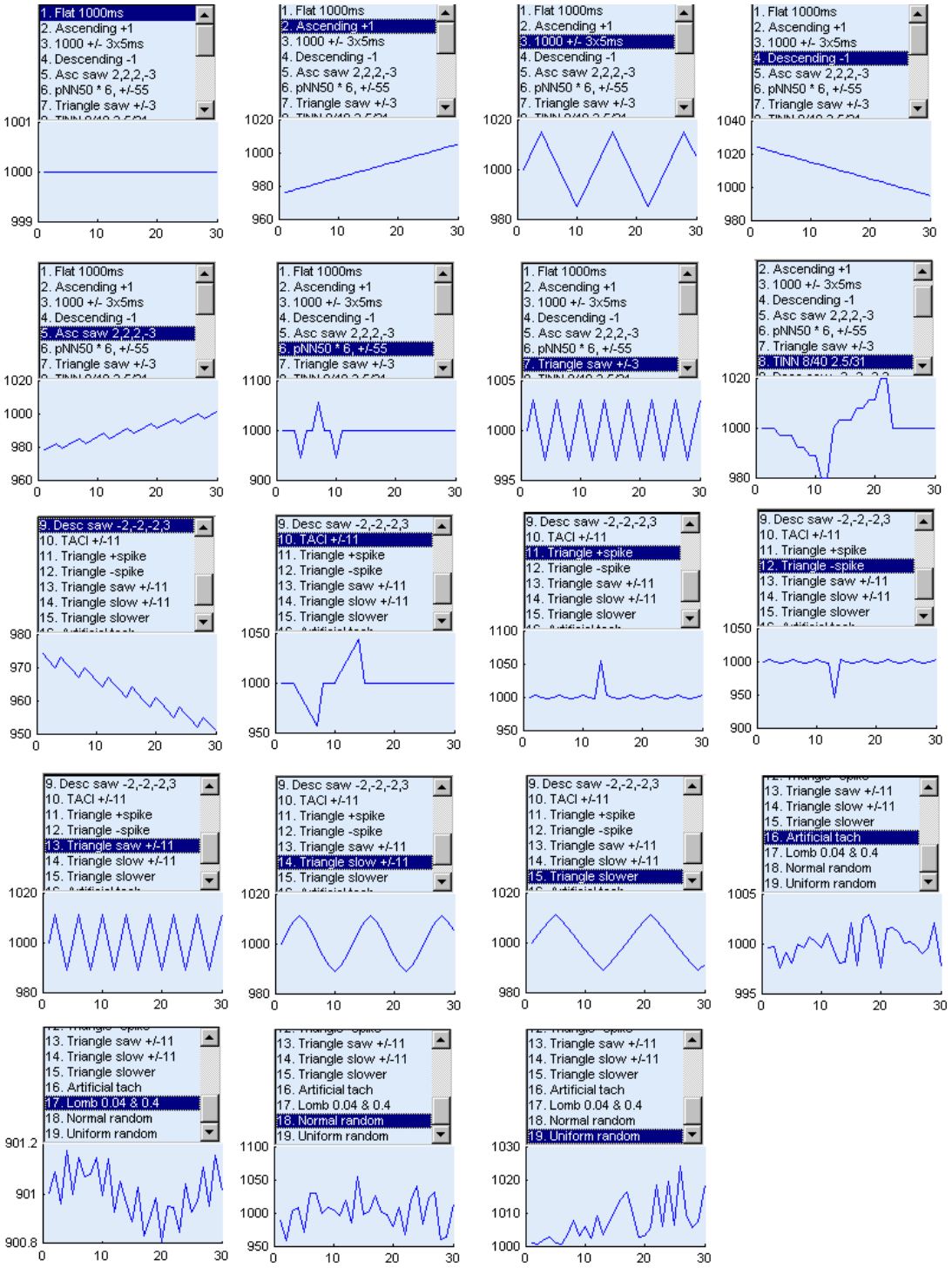


Figure 4-1 Test data developed to check index calculations

4.4.2 Traditional time domain indices

The most commonly used traditional indices (Table 4-1) include the total variability, **SDNN**, and short-term variability, **RMSSD** and **pNN50**. Other time domain indices that could be used with small samples were also investigated: **SDSD**, **NN50**, **TINN8**, **TRIANG8** and the logarithmic index, **DNNEXP**.

Table 4-1 Traditional time domain indices: statistical and geometric, and related indices

Index	First author	Description	Equivalent to
SDNN	Task Force [152]	Standard deviation of NN intervals	
SDANN	Task Force [152]	Standard deviation of the averages of NN intervals in all 5 min segments of recording	Not suitable
RMSSD	Task Force [152]	RMS of successive differences	
SDNN index	Task Force [152]	Mean of the standard deviations of all NN intervals for all 5 min segments of the entire recording	Not suitable
SDSD	Task Force [152]	Standard deviation of NN interval differences	
NN50	Task Force [152]	Number of pairs of adjacent NN intervals differing by > 50 ms	
pNN50	Task Force [152]	Percent of NN intervals >50 ms	
Triang8	Task Force [152]	HRV triangular index, total number of NN intervals divided by the height of histogram in 8 ms bins (1/128 s)	
TINN8	Task Force [152]	Baseline width of triangular interpolation of the highest peak of the NN interval histogram with 8 ms bins	
Differential index	Task Force [152]	Difference between the widths of the histogram of NN interval differences measured at selected height (e.g. at 1000 and 10,000 samples)	Not suitable
DNNEXP	Task Force [152]	Logarithmic index, coefficient φ of the negative exponential curve $k.e^{-\varphi t}$ for histogram of absolute differences NN intervals	
SDNNmc	Antelmi [271]	Mean corrected SDNN	
RMSSDmc	Antelmi [271]	Mean corrected RMSSD	
pNN20	Mietus [272]	Proportion successive NN interval >20ms	
pNN30	Copie [178]	Proportion successive NN interval >30ms	
pNN6.25	Ewing [273]	% successive difference >1/16	

Abbreviations: NN, normal sinus rhythm (traditional abbreviation); RR, the intervals between the R-waves of the ECG (common term); dRR, (delta RR) to the difference between successive RR-intervals.

The use of standard time domain measures to characterise a person's overall HRV status is well documented for time periods from 5 min to 24 hr [152]. **SDSD** is equivalent to **RMSSD** for stationary intervals [179].

Two of the time domain indices, **SDNN** and **RMSSD**, are known to have an inverse correlation with heart rate so were corrected for the mean (**SDNNmc** and **RMSSDmc)** [274, 275].

Pilot analysis showed the **NN50** and **pNN50** were often zero over 30-beat periods so others in the pNNxx family were also included: **pNN20** [272], **pNN30** [178], and **pNN6.25** [273] equivalent to a 50 ms (1/16) change at RR-interval of 800 ms.

The **TINN8** is the baseline width derived from a best least square triangular fit to the main peak of the histogram of RR-intervals with 8ms bins. While the Task Force [152] description of the TINN is straightforward, its actual implementation is not straightforward and the code was not detailed in any studies beyond the statement "minimum square difference used to find the triangle" or "a linear regression was performed for each side of the triangle" [276-282]. Only one published method could be located based on the maximum of the probability function [283].

For this implementation of TINN an approximation was made using the median of the maximum bin values of the histogram as the apex of the triangle. The first approximation for lines of best fit meet well below this point, so repeated iterations increased the height of this point until both lines of least squares best fit passed through the apex (Figure 4-2, more detail in Appendix C).

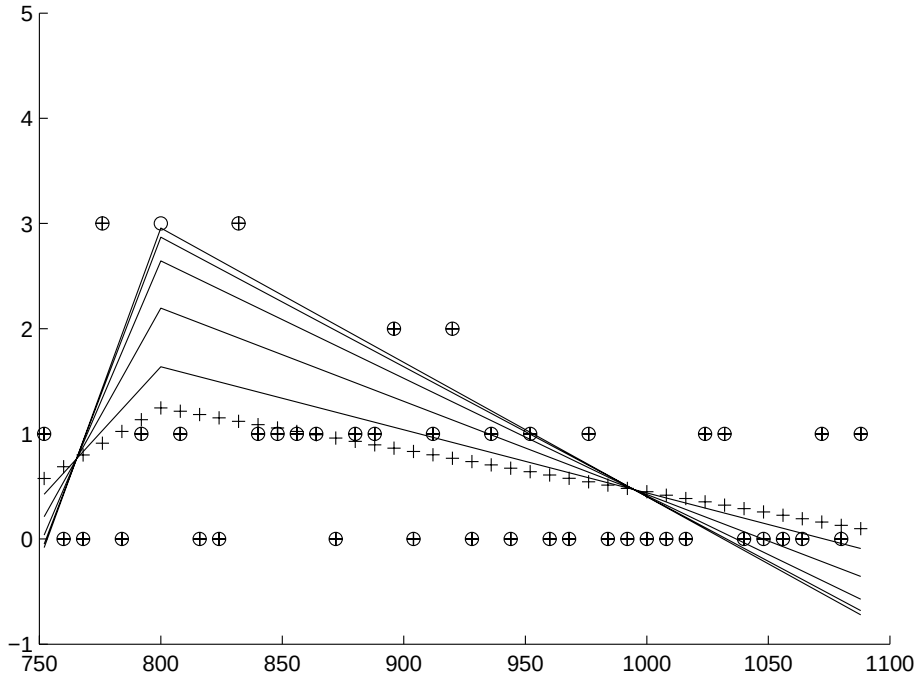


Figure 4-2 TINN calculation example for RR-interval histogram for 30-beat periods with 8ms bins (+ in circle), x-axis is RR-interval time (ms), first approximation of the best fit line (+), repeated iterations (-), and final line intersecting the maximum bin (o).

The logarithmic index, **DNNEXP**, an approximation of the differential histogram by an exponential curve, although recommended for use by the Task Force was not evident in the literature. It may be of limited value for few points. The Task Force was aware of this limitation and recommended a reasonable number of NN intervals (at least 20 min) to construct the geometric pattern.

Three of the traditional indices were not used:

- **SDANN** and **SDNN** both use averages of 5 min data for the 24 hr period so are not useful for short time spans.
- **Differential index** only applies to large numbers (e.g. 86000 beats over 24 hr) [276].

4.4.3 Spectral indices

Spectral power density analysis transforms the RR-interval time series to the frequency domain which is divided into 3 areas for HRV analysis: very low frequency VLF, low frequency LF and high frequency HF. Four methods were assessed for their use over very-short periods.

The nonparametric FFT is the method recommended by the Task Force [152] for spectral analysis with considerable pre-processing of the signal required (i.e. interpolation, detrending, zero padding, and windowing). The unevenly spaced data requires interpolation which introduces inherent low pass filtering, and the need for replacement of outliers [165, 284].

Spectral analysis of HRV requires at least 2-5 min of data (not including determination of very low frequencies, <0.04 Hz) that are known to be stationary. HRV is known to be non-stationary except under controlled conditions. The analysis of dynamic events is challenged by this requirement for stationarity.

The autoregressive method, like FFT, is theoretically defined on an infinite stationary data sequence and introduces errors on finite records by assuming the data outside the window is zero [162]. When data are nonstationary and aperiodic, these analyses may distort the values for frequency and amplitude [285].

The Task Force give further requirements when using the autoregressive technique to specify: the type of the model used, the number of samples, the central frequency for each spectral component (LF and HF), the value of the model order (numbers of parameters), and statistical figures to test the reliability of the model (such as the prediction error whiteness test and the optimal order test to check the suitability of the order of the model used) [152].

The discrete wavelet transform suffers from coarse time-frequency resolution making identification of relevant features within the transform difficult. It also exhibits nonstationarity causing problems in terms of repeatability and

robustness of the analysis unless it can be applied in an ensemble average which is not applicable to the analysis performed here [286].

Although not discussed by the Taskforce [152], the Lomb method is a preferred method [165, 284, 287, 288] for HRV over FFT and autoregressive methods because the RR-intervals are analysed directly, and no attenuation of higher frequencies occurs due to resampling [165]. The Lomb-Scargle algorithm has been used in this study to generate the spectral indices (Table 4-2) and minimise the problems recognised with FFT and autoregression methods. Early testing showed it was feasible to apply the Lomb method to short-term HRV analysis.

Table 4-2 Spectral power indices based on Lomb-Scargle algorithm

Index	First author	Description	Time domain equivalence over 24 hr
LombLF	Moody [284]	Low frequency(0.04-0.15 Hz) power ms^2	
LombHF	Moody [284]	High frequency (0.15-0.4 Hz) power ms^2	RMSSD[152], pNN50[152]
LombVLF		Very low frequency (0.0-0.04 Hz) power ms^2	
LombLFnu	Moody [284]	LF normalised units (over LF+HF)	
LombHFnu	Moody [284]	HF normalised units (over LF+HF)	
LombLF%	Perini [289]	LF as percentage of all power: VLF+LF+HF	
LombHF%	Perini [289]	LF as percentage of all power: VLF+LF+HF	
LombLF/HF	Moody [284]	Ratio of low to high frequency	SDNN/RMSSD [290]
LombTotal	Moody [284]	Total power (LF+HF)	SDNN[152]

MatLab implementation [291] of the fast algorithm for calculating the Lomb-Scargle periodogram [292] was used for analysis (Appendix B.5). The code was modified to return areas associated with peaks at low and high frequencies (**LombLF**: LF 0.04-0.15 Hz and **LombHF**: HF 0.15-0.4 Hz), **LombTotal** total power (LF+HF), normalised LF and HF (i.e. **LombLFnu**: LF/(LF+HF) and **LombHFnu**: HF/(LF+HF)), and the ratio **LombLF/HF**.

Three spectral analysis measures, **LombVLF**, **LombLF%** and **LombHF%**, were not included because the VLF component (0 to 0.04 Hz) is close to DC

and requires a recording at least 10 times the wavelength of the lower frequency bound, so cannot adequately be resolved by even 5 min analysis [293].

The Lomb-Scargle code [291] was shown to be useful with default settings: oversampling factor, ofac of four; and the ratio, hiFAC of one (fhi/fc = highest frequency examined over Nyquist frequency). This allowed spectral power calculations for frequencies up to hiFAC times the average Nyquist frequency (which is the mean heart rate for HRV).

Laguna *et al.* [165] showed that for heart rate variability, the maximum frequency that can be reported on without aliasing is half the Nyquist frequency (i.e. half the frequency of the inverse of the mean heart period); only frequencies up to 0.5 Hz for a 60 bpm heart rate should be considered. A slower heart rate of 50 bpm is still sufficient to identify the upper HF bound of 0.4 Hz.

Although Moody *et al.* were using a Lomb algorithm to identify respiration from the ECG in the 1980's [294, 295] and suggested it be useful for HRV in the early 1990's [284] there have been few studies using any Lomb-derived method for clinical HRV applications. Griffin and Moorman's group [287] have proven it superior to FFT methods in meeting the challenges of neonatal applications in their search to predict neonatal sepsis.

The Lomb-Scargle method has been selected here for the possibility that it is able to provide a spectral measure in very short-term use where other spectral methods are not suitable. There are no publications supporting its use over short-term periods.

4.4.4 RSA, Respiratory sinus arrhythmia indices

Although RMSSD and HF are measures of short-term vagal activity and thus RSA, a range of other RSA indices were also investigated (Table 4-3).

Table 4-3 RSA indices

Index	First author	Description
RSA meanAD	Eckoldt [172]	Mean (corrected for population, 1/(n-1)) of absolute differences over window
RSA medAD	Moser [296]*	Median absolute difference over window
RSA PkValley	Katona [147]	Mean of peak–valley
RSA PVtone	Porges [171]	Vagal tone, 3rd order, 21-point moving polynomial filter
RSA 5RR	Seals [297]	Difference between mean of 5 largest and 5 smallest intervals
RSA 5RRmc	Bergfeldt [298]	Normalised range

* modified from Eckoldt [299]

The respiratory signal is often used in RSA determination to detect the start of inspiration. It can be measured by a number of methods (impedance changes, spirometry, airway thermistor etc.). The respiratory cycles can also be detected in the amplitude of the ECG R-wave; however this requires an ECG lead perpendicular to the electrical axis of the heart, and the posture to be known [300, 301].

The RSA methods relying on knowing the start of inspiration include cosinor analysis [302, 303], voluntary cardiorespiratory synchronisation [304], phase synchronisation [305], fractional cardiac cycle count [306], and polar or circular statistical analysis [201, 235, 246, 307, 308]. These methods are not suitable for the databases used because respiratory signal data was not available and ECG axis was unknown.

The RSA measurements that were used in this study are all suitable for use over short periods and only require the RR-interval data:

1. Range: **RSA 5RR**, **RSA 5RRmc**
   The simplest range measure of respiratory variations is the difference in maximum and minimum heart period, VHP, reported by Katona and Jih [147] defined here as RSA. Katona *et al.* [309] found it to be

proportional to parasympathetic control in dogs. This finding was replicated in people by Fouad *et al.* [310] but using the difference between the average of all maximums and all minimums of a 30 s window. Seals *et al.* [297] used the difference between average 5 maximums and average 5 minimums and this has been used here (**RSA 5RR**).

The nonlinear relationship between heart rate and RR-intervals causes a higher peak-valley measure of HRV for a slower heart rate when it is the same HRV [275]. A normalised range (RSA 5RRmc) was included as used by Persson and Solders [311] and Bergfeldt *et al.* [298]. Heart rate variations (max-min) as a % of mean RR-interval [311] have been used for assessing autonomic dysfunction in Guillain-Barré syndrome [312] and sinus node dysfunction [298].

2. Peak-valley: **RSA PkValley**

   The mean of all the peak-valleys (absolute difference between local maxima and minima) within the measurement period was measured within the 30-beat period rather than the respiratory cycle. The maximum of the local maxima was not used as it tends to bias toward large sighs and breaths that may be artifacts [147, 244, 297, 313, 314]. The peak valley measure is highly sensitive to variations in tonic parasympathetic cardiac control, but can be confounded by slower heart rate variations (i.e. 0.03-0.1 Hz) [170]. To ensure slower breathing rates were included, the signal was not pre-filtered to limit to normal respiratory frequencies 0.15-0.4 Hz as others have done [171].

3. Porges vagal tone: **RSA P.Vtone**

   This method has been widely used since it was patented by Porges in 1985 [171, 234, 315] and used in the Vagal Tone Monitor (Delta-Biometrics Inc., Bethesda, MD). It monitors dynamic changes over a short period by using a moving third order 21 point polynomial filter at 500 Hz to encompass 10.5 s. This produces frequencies from 0.15 to 0.4 Hz covering normal adult breathing rates 9-24 $br{\cdot}min^{-1}$. This was implemented in MatLab with the SMOOTH function in the

curve fitting toolbox. A Savitzky-Golay filter (generalised moving average with filter coefficients determined by an unweighted linear least-squares regression and a polynomial model of specified degree) was used with a window length of 21 and a polynomial fit order of 3.

4. Mean absolute difference: **RSA meanAD**
   Eckoldt measured absolute sinus arrhythmia as the mean of the absolute differences between successive beats within the window [172, 299]. (The actual German word used by Eckoldt was “mittlere” which can be translated as “mean”, “average”, “middle” or “median”, but the equation used 1/ (n-1) times the sum of the differences, that is the mean.) Hayano *et al.* found the mean successive difference a good measure of assessing vagal tone with pharmacological blockade [197]. Bockelmann *et al.* used this method over periods of >200 beats to measure vagal depression in long-term lead (i.e. Pb) exposure [313]. The average absolute difference, AAD, is available in a commercial device (Neuroscope, MediFit Diagnostics Ltd, London) as a cardiac index of parasympathetic activity, CIPA, over 5 min has been proven to be quantifiable with 10 s recording [210], however, the 10 s AAD was not proven to be useful for studying mortality in old age [316].

5. Median absolute difference: **RSA medAD**
   Moser *et al.* [173] used the median of absolute differences between beats, rather than mean, with no comment on this selection. The median absolute difference was preferred to the mean absolute differences being a better indicator of the middle of a skewed selection of values. Use of the median should reduce the effect of outliers. They were looking for autonomic nervous system changes in depressed subjects and used a logarithm transform of this index to enable statistical tests.

4.4.5 Poincaré plot indices

The Poincaré plot, where each beat is plotted against the succeeding beat, has produced a wealth of indices that were originally thought to be nonlinear [180, 184, 185], but were later shown to be scaled versions of time domain indices [179].

Some Poincaré plot indices that were impractical for coding with a small number of beats included: HRV fraction [317], a 24 hr density measure; visual pattern analysis [181-183, 192]; measurements of dispersion within the Poincaré plot [183-185, 187]; and ellipse fitting [178, 180, 185, 186, 204, 318]. The problem of fitting ellipses to small samples can be seen going from 100 beats (Figure 4-3) to 25 beats (Figure 4-4).

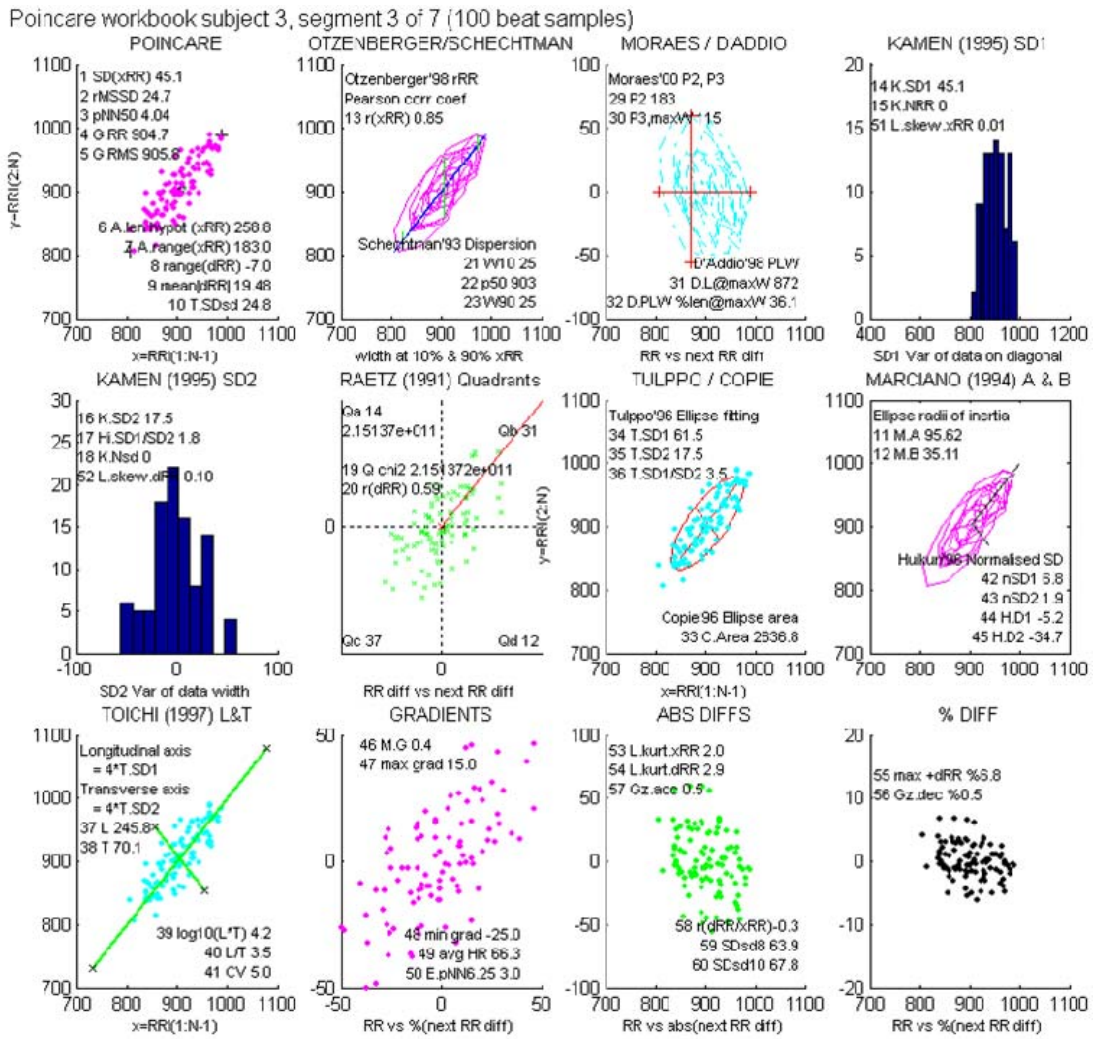


Figure 4-3 Example of Poincaré plot index calculations with 100 beats

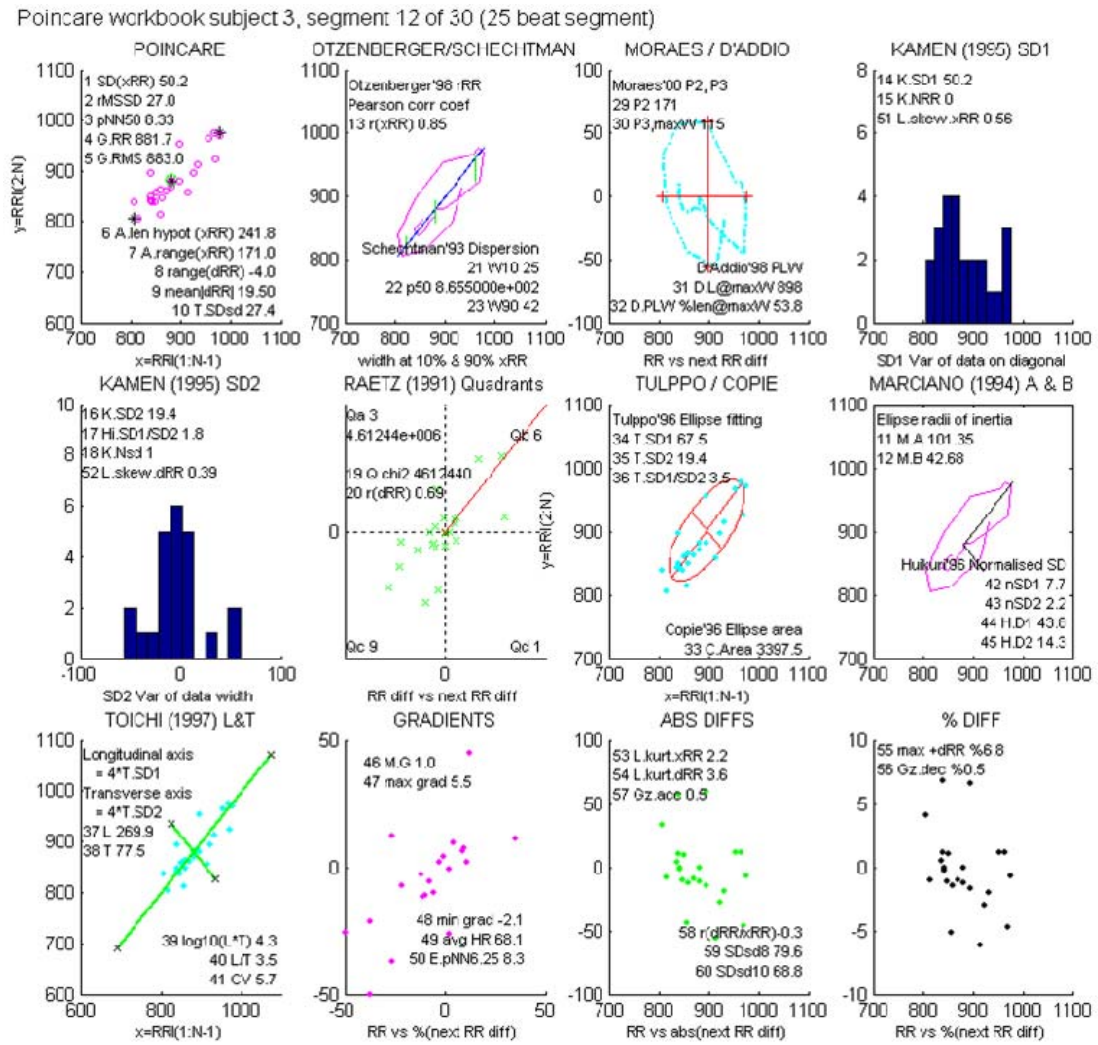


Figure 4-4 Example of Poincaré plot index calculations with 25 beats showing problems with small samples and ellipse fitting

Density was not measured in any of its different forms: compactness (log integral of density function) [188]; maximum plot wideness [185]; % length at maximum width [184]; 80% wideness at 10th and 90th percentile (i.e. at slow and fast HR) [183]; scanning parameters [186]; or 3D characteristics [187]. Density and 3D measures were not appropriate for small numbers being originally designed to characterise large amounts of data such as 90,000 beats collected over 24 hr, when multiple points are closely clustered and cannot be distinguished. Recurrence [319, 320] was not investigated here as it is usually being analysed over 200-1000 cycle (breath or beat) segments.

The following Poincaré plot indices were used (Table 4-4):

1. Asymmetry: **accel**, **decel**, **assym(R/L)**
   The Poincaré plot is asymmetrical about line of identity [321, 322]. The Poincaré plot width components can be separated out into decelerations (above line of identity) and accelerations (below line of identity). The MatLab code for **accel** and **decel** was detailed by Piskorski and Guzik [322]. Another method by Kovatchev *et al.* sample asymmetry analysis (SAA), defined here as **assym(R/L)**, analyses the histogram tails, exaggerating points away from the median caused by transient accelerations and decelerations [323].

2. Quadrants: **pQa**, **pQb**, **pQc**, **pQd**
   A plot of the successive differences between RR-intervals can be separated into four quadrants with preference based on autonomic nervous system control and asymmetric actions of the sympathetic and parasympathetic nervous systems [202]. The top right hand quadrant has the highest score for runs of increasing RR-intervals, and bottom left hand for runs of decreasing RR-intervals. The original study by Raetz *et al.* used a chi-squared test to determine significance of the distribution in different sleep stages [202]. Here the proportion in each quadrant has been reported.

3. Length and breadth: **SD1**, **SD2**, **SD1nu**, **SD2nu**
   Many of the length and breadth measures of the Poincaré plot (SD1, SD2, L, T or W, A, B) are actually linear measures that are scaled versions of SDNN and RMSSD [179] so these time domain equivalents were used in their place. The short-term variability, **SD1**, is equivalent to a scaled SDSD [179]. The length of the Poincaré plot, **SD2**, is a better measure of long-term variability being equivalent to the total variability (SDNN) less the short-term variability (SD1) [179].

   The following were replaced by the aforementioned method: manual measurement of axis lengths [178], radii of fitted ellipses SD1 and SD2 [184, 185, 324], ellipse rotated ±45 degrees in polar coordinates [180],

maximum and minimum radii of inertia (A, B) [186],and others that only stated length and width of transverse and longitudinal axes, L and T [204, 318]. Also included in this study are the normalised measures, SD1nu and SD2nu (divided by mean RR-interval) used by Huikuri *et al.* [185].

4. Ratios of length and breadth: **CSI**, **SDNN/RMSSD**, **SDNN/SDSD**
   Ratios of the length and breadth, SD1/SD2 [180, 185], SD12 [324] and L/T [318] also called the cardiac sympathetic tone index, **CSI**, by Toichi *et al.* [204], were modified to use the time domain indices as described by Brennan *et al.* [179].
   The **SDNN/RMSSD** ratio was used by Balocchi *et al.* for periods as short as 64 s [290]. The Hirose *et al.* [325] ratio of SDNN to SDSD was also used as SDSD is only equivalent to RMSSD in stationary conditions [179]. The ratio of short to long term variability, also called SD1/SD2 [180, 185], was not included.

5. **area**
   The cardiac vagal tone index, CVI, developed by Toichi *et al.* is the product of the axes length and width, $\log_{10}(L \cdot T)$ [204]. Rather than using the ellipse axes of the original, this measure was converted to time domain measures [179]. This index was used for all other area indices: L·T [318], $4 \cdot pi \cdot SD_{Long} \cdot SD_{Short}$ [282] and 0.25·pi·L·W [178]. 3D area, M·N, was not included [187].

6. Autocovariance: **r(RR)**, **acv0x**
   The interbeat autocorrelation coefficient (or normalised autocovariance), **r(RR)**, is equivalent to the Pearson correlation coefficient between RR(n+1) and RR(n) and has indicated sympathovagal balance, LF/HF, in sleep studies [205]. The first zero crossing of the autocovariance, **acv0x** or decorrelation time [162], was noted by Sosnowski *et al.* for its relationship to RSA that disappeared with atropine [326].

7. Lagged plots: **SDLD4**, **SDLD8**, **SDLD10**, **mean r(L1-6)**
   Lagged plots extend the number of beats plotted against RRn to RRn+1, RRn+2 etc. For each of these lagged plots Poincaré plot indices can be calculated e.g. SD1, SD2, SD1/SD2, SDLD [209, 327, 328].

   Lerma *et al.* [327] found (investigating renal failure) a lag of 4 for the short to long term variability ratio, T/L, reflected nonlinear information not related to linear autocorrelations. Contreras *et al.* [328] note that plot width SD1 is correlated with HF and decreases as lag increases, and that after lag 4, plot widths correlated with LF with a plateau after lag 8. Plot width SD1 for lagged plots was calculated using standard deviation of lagged differences **SDLD** for lags 4, 8 and 10 (even though the recommended maximum lag is one fifth of the window length [329]). Thakre and Smith [209] used **SDLD** to show subjects with congestive heart failure had no change for lags from one to ten, with normal subjects reaching plateau at lag eight.

   Sosnowski *et al.* [326] calculated correlation coefficients, r, for each plot from one to 25 lags for records > 500 beats, then assessed a variety of indices: r(1), r max, r(1)-r(25), r max-min, and **mean r(L1-6).** The last was found to be an indicator of RSA.

8. Central tendency: **CTMdRR**
   Cohen *et al.* [330] plotted the second order difference plots and used a measure of central tendency to describe the tightness of the distribution. They found that congestive heart failure could be identified by a wider spread. Cohen used different thresholds of radius to characterise dispersion. In this study, the inverse of normalised area was used as the measure of central tendency.

Table 4-4 Poincaré indices

First author	Index	Description	Equivalent to index
Balocchi [290]	SDNN /RMSSD	Ratio similar to LF/HF and SD2/SD1, sympathovagal balance	
Brennan [179]	SD1	SD of ellipse width equivalent to SDSD scaled by 0.707, short-term variability	SDSD
Brennan [179]	SD2	SD of ellipse length, in time domain terms: $(2*SDRR^2-0.5*SDSD^2)^{0.5}$, long-term variability (or total less short-term variability)	
Brennan [179]	SD1nu	Equivalent to normalised SD of ellipse width	
Brennan [179]	SD2nu	Equivalent to normalised SD of ellipse length	
Brennan [179]	area	log(SD1*SD2) using Brennan definitions for SD1 and SD2	
Brennan [179]	ratio	SD2/SD1 using Brennan definitions for SD1 and SD2	
Carrasco [318]	L*T	Area of ellipse – not suitable	Brennan area
Carrasco [318]	L,T	Radii of fitted ellipses L and T, not suitable	Brennan SD1, SD2
Carrasco [318]	L/T	Ratio of fitted ellipses L and T, not suitable	Brennan ratio
Cohen [330]	CTMdRR	Central tendency measure	
Contreras [328]	SDLD4 SDLD8 SDLD10	Lag of 4,8 and 10 beats for SD1 of differences	
Copie [178]	A	Area of ellipse, $pi{\cdot}L{\cdot}W{\cdot}4^{-1}$ – not suitable	Brennan area
Copie [178]	L, W	Manual measurement of axis lengths	Brennan SD1, SD2
D'Addio [184]	%LmaxW	% length at maximum width	Not suitable – density
D'Addio [192]	pattern	Pattern analysis: comet, torpedo, fan, or complex	Not suitable – pattern
D'Addio [184]	SD1, SD2	Radii of fitted ellipses SD1 and SD2	Brennan SD1, SD2
Guzik [321] from Piskorski [322]	accel decel	Asymmetry – accelerations Asymmetry – decelerations	

First author	Index	Description	Equivalent to index
Hnatkova [188]	compactness	Log integral of density function	Not suitable – density
Hirose [325]	SDNN /SDSD	Ratio SDNN to SDSD	
Huikuri [185] modified by Brennan [179]	SD1nu	Normalised SD1, SD1/meanRR*100	Brennan SD1nu
	SD2nu	Normalised SD2, SD2/meanRR*100	Brennan SD2nu
Huikuri [185]	STD1/STD2	Ratio of fitted ellipses	Brennan ratio
Huikuri [185]	STD1, STD2	SD of instantaneous and long-term continuous RR-interval variability measured from axis	Brennan SD1, SD2
Huikuri [185]	W max thick dist	Distance between centroid and averaged max instantaneous RR-interval variability	Not suitable – density
	Max thick dist	Distance between centroid and maximum instantaneous RR-interval variability	Not suitable – density
Javorka [320]	recurrence	Recurrence quantification analysis: % Det, L_{max}, TT, *Lam*, V_{max}	Not suitable – small samples
Kovatchev [323]	assym (R/L)	SAA, sample asymmetry analysis	
Marciano [186]	PE PP	Scanning parameters on vertical line from the origin to the point of maximum extension: PE plot extension, and PP position of peak as a percent of PE	Not suitable – density, ellipse
Marciano [186]	A, B	Maximum and minimum radii of central ellipse of inertia	Brennan SD1, SD2
Marciano [186]	distribution	Number of peaks, NP, and their average distance from the diagonal line, DP	Not suitable – density
Moraes [187]	MN	3D volume: MN is the product $P_1*P_2*P_3*10^{-3}$. Where P_1 is the mean slope at maximum density, P_2 is the maximum longitudinal range and P_3 the maximum transversal range	Not suitable – density
Moraes [187]	P_2, P_3	P_2 is the maximum longitudinal range and P_3 the maximum transversal range	Not suitable – density
Nikolopoulos [162]	acv0x†	Lag of auto covariance first zero crossing	
Otzenberger [205]	r(RR)	Interbeat autocorrelation coefficient, nonlinear estimate of RSA	
Raetz [202]	pQa pQb pQc pQd	Proportion of sequences by quadrant: a: decreased RR-interval then increase, b: increase followed by increase, c: decrease followed by decrease, d: increase followed by decrease	

First author	Index	Description	Equivalent to index
Schechtman [183]	dispersion	80% wideness at 10^{th} and 90^{th} percentile (i.e. at slow and fast HR)	Not suitable – density
Schechtman [183]	scatter	Range between 10^{th} and 90^{th} percentile of RR-intervals	Not suitable – density
Sosnowski [317]	HRVF	HRV fraction is the two highest counts in scatter plot histogram differing from the consecutive beat by <50 ms	Not suitable – density
Sosnowski [326]	r(1)	Correlations of lagged plots with lags from 1 to 25	Otzenberger r(RR)
Sosnowski [326] modified by Brennan [179]	mean r(L1-6) r(1)-r(25) r max r max-min	Mean of correlation coefficient for return maps with lags 1-6, estimate of RSA Difference of correlations for return maps with lags 1 and 25 Maximum correlation for return maps with lags 1 to 25 Difference of max and min correlations for return maps with lags 1 to 25	
Stein [324]	SD12	Ratio of long (SD2) to short (SD1) axis	Brennan ratio
Toichi [204]	CSI	Cardiac sympathetic index L/T, longitudinal over transverse axis	Brennan ratio
Toichi [204]	CVI	Cardiac vagal index, area is log (L*T). Original form not suitable - ellipse	Brennan area
Toichi [204]	L, T	Length of longitudinal and transverse axis	Brennan SD1, SD2
Tulppo [180]	SD1, SD2	SD of horizontal axis for fitted ellipse rotated + and - 45 degrees	Brennan SD1, SD2
Tulppo [180]	SD1/SD2	Ratio of SD for fitted ellipse radii	Brennan ratio
Webber [319]	recurrence	Recurrence analysis: Shannon entropy and upward diagonal lines, %recurrence, and %determinism	Not investigated
Woo [181, 182]	pattern	Pattern analysis: comet, torpedo, fan, or complex	Not suitable - pattern
Ziegler [282]	area	Area of ellipse $4*pi*SD_{Long}*SD_{Short}$	Brennan area
Ziegler [282]	SD_{Long}, SD_{Short}	SD of long axis and short axis	Brennan SD2, SD1
Ziegler [282]	$CVSD_{Short}$ $CVSD_{Long}$	Coefficient of variation for SD of short axis and long axis: SD/meanRR*100	Brennan SD1nu Brennan SD2nu

Abbreviations: SD, standard deviation.

4.4.6 Other indices

Many other indices have developed from statistical measures of heart rate characteristics. Although they were not actively surveyed, some nonlinear indices were found and were applicable to very short time analysis so they were included.

Changes in heart rate with cardiovascular reflex tests, before and after a brief impulse, were also not considered: Valsalva manoeuvre with forced breathing for 20 s against an expiratory pressure of 40 mmHg [331, 332]; response to standing [333]; turbulence onset with ventricular beats [334-336]; simulated diving apnoeic facial immersion in cold water [337, 338]; or carotid baroreflex stimulation with neck suction or pressure [297, 339].

Other identified indices that could be measured for periods of <1 min (Table 4-5):

1. Sign and magnitude : **sign(dRR)** and **magn(dRR)**
   Ashkenazy *et al.* developed scaling exponents of short to long range (n = 6 to 1024 beat) correlations of 6 hr periods [340] and used it to distinguish sleep stages [341]. This detrended fluctuation analysis has been simplified to use the basic forms separately: sign and magnitude.

2. Threshold-based acceleration change index: **TACI(10)**, **TACI(20)**
   Arif and Aziz used a sign series similar to Ashkenazy *et al.* but generated relative to a threshold; the crossings of the threshold are characterised by TACI [342]. This is based on a non-threshold measure developed by Gonzalez *et al.* [343] The original study on 10 hr recordings used a threshold of 50 ms, longer than the 10 and 20 ms thresholds found useful here with the shorter time period.

3. Coefficient of variation: **CVRR**, **CVdRR**
   The coefficient of variation (i.e. the ratio of standard deviation to mean), minimises the effect of heart rate on variability.
   Hayano *et al.* [197] showed the CV to be correlated with atropine controlled vagal activity when sympathetic activity was removed by propranolol. Van Hoogenhuyze *et al.* [344] found the index was

reproducible over 2 days using averaged 5 min CVs of a 24 hr period. Toichi *et al.* [204] tested this index with atropine (which blocks parasympathetic activity) over 3 min but found only mild changes. Some authors had no significant findings of this variable over periods from 3 to 5 min [345-348]. Tateno and Glass [349] used **CVRR** and **CVdRR** for characterising atrial fibrillation.

4. Normality: **normRR**, **norm.dRR**
   Tateno and Glass [349] measured the largest difference of the cumulative probability distribution to a standard distribution using a Kolmogorov-Smirnov test for characterising atrial fibrillation over periods from 20 to 200 beats. They found 100 beats gave the optimal performance. In this study, the Lilliefors statistic was used for its better ability to test normality in small samples [350].

5. Moments: **skewRRz**, **skew.dRR**, **kurtRRz**, **kurt.dRR**
   Central moments (about the mean) include third and fourth moments: skew [349, 351, 352] and kurtosis [353, 354] also referred to by Olesen *et al.* [354] and Lewkowicz *et al.* [353] in multipole terms as octupole (skew along x- and y- axis) and hexadecapole moments (ratio of kurtosis along y-axis to x-axis). The standard MatLab commands for skew and kurtosis were used after normalising data as done by Griffin and Moorman [189] by the mean and standard deviation of each data block.

6. Root mean square residual: **RMS**
   The measure of the deviation of RR-intervals from a straight line has been directly related to parasympathetic activity reactivation after exercise when measured over short periods of 15, 30 and 60 s [355].

7. Mean and median RR-interval: **meanRR**, **medRR**
   The mean RR-interval has been shown to be a surrogate measure of sympathovagal balance [152, 356]. Griffin and Moorman [189] used the median, the 50^{th} percentile data point, as one indicator of sepsis or sepsis-like illnesses in new born infants.

8. Gradient: **gradRR**, **grad5max**, **grad5min**

   The average gradient **gradRR**, the rate of change of RR-intervals, was used without comment by Marciano *et al*. [186].

   Turbulence slope, a measure originally used within 20 beats of a ventricular premature beat, is the maximum slope of a regression line over 5 beats [334, 335] described here as **grad5max**. The minimum slope was also included as **grad5min**.

9. Histogram widths: **SDlen**, **SDwid**

   SDlen is the histogram variance of RR, which is the same as the SDNN. **SDwid** is histogram variance delta RR, around the line of identity, similar to **RMSSD** if mean dRR = 0, a parasympathetic measure. The histogram methods [177, 203] are equivalent to the length and transverse axis of an ellipse, but are easier to calculate (particularly as the sample size decreases) [179].

10. Low variability: **PolVar20**

    Probability of low variability for 6 beats in a row, originally abbreviated to plvar20 [357], characterises intermittent phases of low variability from successive RR-intervals, in this case <20 ms. With a threshold of 10 ms, plvar10 was found to be useful in conjunction with other parameters in risk stratification for acute myocardial infarction [358]. Later, Wessel *et al.* used the renamed POLVAR10 and POLVAR20 for identifying congestive heart failure [359], and for forecasting ventricular tachycardia [360].

11. Variability Index: **VarIndex**

    The mean percentage of differences between two adjacent RR-intervals divided by the second RR-interval was found by Copie *et al.* [178] to be equivalent to scatter plot width and **pNN50** in patients with recent myocardial infarction.

Table 4-5 Other indices

Index	First author	Description	Equivalent to
CVdRR	Tateno [349]	Coefficient of variation of dRR	RMSSDmc
CVRR	Van Hoogenhuyze [344] Toichi [204]	Coefficient of variation of RR-intervals, std/mean*100	SDNNmc
gradRR	Marciano [186]	Gradient RR-intervals (average), DR	
grad5max	Schmidt [335]	Turbulence slope, max gradient of 5 beats	
kurtRRz	Olesen [354] Griffin [189]	Kurtosis, peakiness of histogram, normalised	
kurt.dRR	Olesen [354]	Kurtosis, histogram peakiness	
magn(dRR)	Ashkenazy [340]	Magnitude of differences	
meanRR	Goldberger [356]	Mean RR-interval, sympathovagal balance	
medRR	Griffin [189]	Median RR-interval, sympathovagal balance	
normRR norm dRR	Tateno [349]	Difference of distribution to normal measured by Kolmogorov-Smirnov or Lilliefors test statistic	
PolVar20†	Voss [357] Wessel [359]	Probability of low variability, <20 ms difference for 6 beats in succession	
RMS	Goldberger [355]	Deviation of RR-interval from straight line	
SDlen	Kamen [177]	Width of RR-interval histogram	Brennan SD2
SDwid		Width of delta RR histogram	Brennan SD1
sign(dRR)†	Ashkenazy [340]	Sign of differences	
skew.dRR	Tateno [349]	Asymmetry RR-interval differences	
skewRRz	Griffin [189]	Asymmetry of histogram (negative has left tail), normalised	
TACI(10)† TACI(20)†	Arif [342]	Threshold based acceleration index with 10 or 20 ms thresholds	
VarIndex	Copie [178]	Mean % difference between RR-interval divided by the next interval	

4.5 Discussion

4.5.1 Selection of indices

This survey identified a selection of indices beyond the standard time and spectral domain indices that may be useful in short-term analysis of autonomic nervous system activity. The indices included less well known measures of heart rate fluctuations and characteristics.

4.5.2 Measures of sympathetic and parasympathetic activity

The 1990's produced a wealth of HRV indices in the quest for better measures of parasympathetic and sympathetic activity.

The two commonly used time domain indices, **SDNN** and **RMSSD**, measure the total power of variation and vagal tone respectively. These indices are simple to calculate and are recognised over longer periods (2-5 min) as surrogates for many other indices that are difficult to measure, including frequency domain indices [179, 318]. This correlation may not be apparent over the very short-term (< 1 min). Indices that do not correlate strongly with **SDNN** or **RMSSD** over a variety of physiological states may prove useful in determining dynamic characteristics of cardiac nervous system activity.

Many of the surveyed indices identify changes in parasympathetic activity for longer record lengths: **RMSSD**, **pNN50**, **HF** power and all **RSA** indices.

There are no indices that are reliably used for measuring sympathetic activity [361]. The ratio of spectral power, **LF/HF**, is used as a measure of sympathovagal balance and usually needs 2-5 min stationary periods for effective analysis.

4.5.3 RSA indices

RSA indices are alternative measures of parasympathetic activity related to respiration. Only included were those **RSA** indices that were based on heart rate data and that did not require respiratory signals.

4.5.4 Poincaré plot issues

While the Poincaré plot is a nonlinear method, many of the indices have been shown to relate to time domain indices [179], so these were used where they were shown to be equivalent. Many Poincaré plot indices were shown to be unsuitable, especially those fitting ellipses to the data or measuring density of the distribution.

4.5.5 Other indices

The indices in this group included heart rate characteristics rather than typical HRV measures, and excluded those related to applied impulses. While nonlinear analysis was not specifically included in this survey, some nonlinear indices appropriate for short-term analysis have been included.

4.5.6 Limited use

Many of the lesser known indices have had limited use in specific studies, for example, **CTMdRR** was used by Cohen *et al.* identifying congestive heart failure [330], **acv0x** was characterised by Sosnowski et al. with atropine [326], and **RMS** was used by Goldberger *et al.* for examining parasympathetic reactivation after exercise [355]. These indices have not been investigated more widely to determine their usefulness in normal physiological states.

4.5.7 Problems of spectral analysis

Window size

It is generally recommended for HRV that spectral analysis be performed on a recording at least 10 times the wavelength of the lower frequency bound, that is at least 2 min (256 to 512 beats) for the LF component [152], to ensure sufficient frequency resolution while maintaining stationarity.

Contamination

Two problems have been identified with the Lomb method. Hyndman and Zeelenberg [362] show it introduces bias into the HRV spectrum, and it introduces a degree of nonlinear distortion with the spectral power of two output components of a simulation altered by the addition of a third sine to the input. Laguna *et al.* [165] show that in extreme situations (low heart rate or high-frequency components) the Lomb estimate introduces high frequency

contamination. Furthermore, spectral analysis of non-stationary heart rate by any method will produce distortions described by Laguna *et al.* [165] as an increase in low frequency power artifact that is unrelated to HRV.

4.6 Summary

This study identified indices published through the 1990s to the present that may be useful for analysis of the autonomic nervous system over short-term periods, < 60 s.

The next chapter will investigate: a) the minimum window length that produces reliable spectral power, and b) spectral contamination in these windows.

Chapter 5. Determination of minimum window size for spectral indices

5.1 Aim

The focus of this thesis of monitoring the dynamic autonomic nervous system responses to the opioid fentanyl up to the peak effect in 5 min [72] requires analysis over windows shorter than the commonly used 2 min in order to detect changes during development of respiratory depression.

While many time domain and statistical indices are able to be calculated over small windows, spectral power density places specific demands on the length of record being investigated. Tests were made to determine the smallest window size that could still produce valid spectral data.

Tests focused on the spectral power density calculated with the Lomb-Scargle algorithm to determine a) if windows < 300 beats could produce useful results, b) what the minimum window length could be, and c) what side-effects could occur.

5.2 Background

5.2.1 Spectral analysis requirements

The Task Force [152] recommend for HRV that spectral analysis be performed on a recording of at least 10 times the wavelength of the lower frequency limit, and to ensure stability, not extend substantially beyond this. The Task Force go on to state required periods of approximately 1 min for the HF component and 2 min for the LF component[5]. The range of frequencies (Table 5-1) for LF is 0.04-0.15 Hz (25 to 7 s) and HF to 0.4 Hz (2.5 s). The third range VLF, from DC to 0.04 Hz, is not associated with parasympathetic or sympathetic activity [152], and so will not be specifically investigated here.

[5] Note: The Task Force recommendations are confusing as the lower limit of LF at 0.04 Hz requires 250 s (i.e. 10/0.04) which is 4.17 min, twice as long as the 2 min recommendation they make. A 2 min record length would only capture a lower limit of 0.083 Hz.

Table 5-1 Frequency characteristics for spectral density analysis

	Frequency range Hz	Cycle length s	Recommended length minute
VLF	0-0.04	>25	>5
LF	0.04-0.15	25-7	2-4
HF	0.15-0.4	7-2.5	1

5.2.2 Bias, distortion and contamination

The Lomb method recommended for spectral analysis of HRV has had two problems identified with it. Hyndman and Zeelenberg [362] comment that it introduces spectral bias into the HRV spectrum with low pass filtering, and it introduces a degree of nonlinear distortion with the spectral power of two output components of a simulation altered by the addition of a third sine wave to the input.

Laguna *et al.* [165] showed that in extreme situations (low-heart rate or high-frequency components) the Lomb estimate introduces high frequency contamination. Furthermore, spectral analysis of non-stationary heart rate by any method will produce distortions described by Laguna *et al.* [165] “as an artifactual increase in low frequency power that is unrelated to HRV”.

These issues with the Lomb method will be investigated here.

5.3 Method

5.3.1 Resolution of outer limit peaks

The first test was to determine the minimum window that could resolve the spectral peak amplitude with significance p = 0.05. Two peaks were selected at the outer limit of frequencies used in HRV analysis, 0.04 and 0.4 Hz. Frequencies with related harmonics (common multiples) can cause interactions, so the frequencies 0.041 and 0.37 Hz were used for the outer limit analyses. Windows with decreasing record lengths (300, 50, 30, 27 and 25 beats) were used, with a typical resting mean RR-interval of 950 ms.

The synthesised signals were created using the equation:

$$rri = mRRI + a_1 \sin(2\pi\omega_1 t) + a_2 \sin(2\pi\omega_2 t)$$

where: *rri* is the RR-interval generated in s; *mRRI* is the mean RR-interval in s; a_1, and a_2, are the amplitudes of the sine waves, both 0.06; ω, and ω_2 are the two frequency peaks at 0.041 and 0.37 Hz; and *t* is time in s. Each set started with a random offset of *mRRI* by ±5 ms, and each iteration incremented *t* by the new *rri*.

An example of the synthesised signal with outer limit peaks is shown in Figure 5-1.

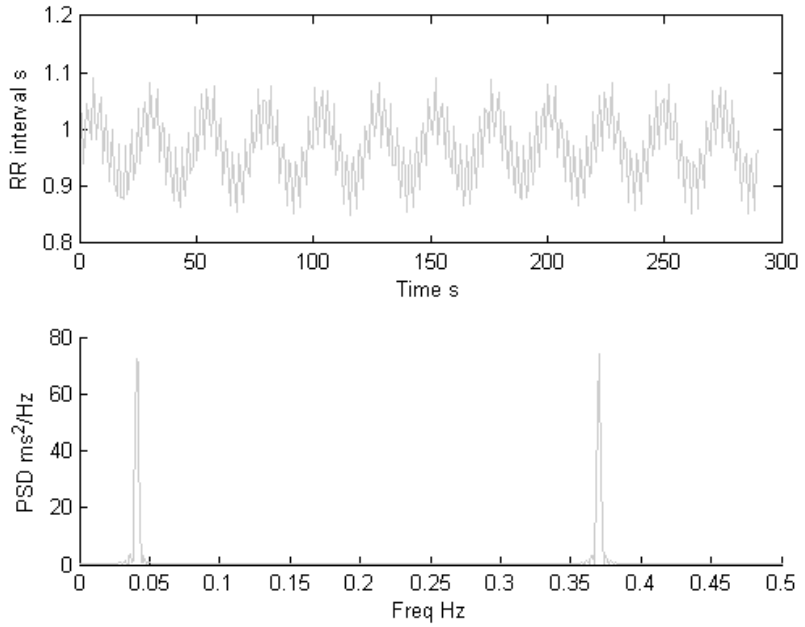


Figure 5-1 Synthesised signal with outer limit peaks at 0.041 and 0.37 Hz: 300 beats at mean RR-interval of 975 ms (top) and spectral power (bottom)

5.3.2 Resolution of outer limit peaks for decreasing RR-intervals

The use of beats, rather than time, to set the window length means that the time covered by the window changes with mean heart rate (bpm) or mean RR-interval (ms) (e.g. with a mean RR-interval of 975ms, a 100 beat window takes 97.5 s, but only 50 s with a mean RR-interval of 500ms).

The test for determining the minimum window for resolution of spectral peak amplitude at outer limits was repeated for decreasing mean RR-intervals down to 500 ms typical of exercise.

5.3.3 Spectral power of outer limits for decreasing mean RR-intervals

The spectral measure of interest to HRV is the power density in $ms^2 \cdot Hz^{-1}$ (i.e. the area under the spectral power vs. frequency curve). Four power measures were investigated:

- LF, low frequency 0.04 to 0.15 Hz.
- HF, high frequency 0.15 to 0.4Hz.
- TP, total power 0.04 to 0.4 Hz (i.e. LF + HF).
- AP, all power 0 to 0.4 Hz (includes VLF component).

The VLF component is included within AP to identify contamination effects. Typically it measures cycles > 25 s so it should have limited effect on the power in short windows.

Window lengths from 600 beats down to 25 beats were used for these analyses. The longer 600 beat window covers a period of 5 to 10 min depending on the heart rate to allow comparison with more commonly used window lengths.

The mean (and SD) of multiple repetitions (1000x) were used to show the effect of reducing window size on these power measures using outer limit peaks (0.041 and 0.37 Hz). The synthesised waveform created with outer limit peaks is atypical and not indicative of normal HRV signals.

5.3.4 Validation with realistic synthesised data

To create a more realistic waveform, four databases of different physiological states archived on PhysioNet [363] were examined to identify typical HRV characteristics (Table 5-2): mean RR-interval; SDNN, standard deviation of the RR- (or NN) intervals; and SDSD, standard deviation of successive differences of the RR-intervals. Full details of Resting, Sleep, Meditation and Exercise databases are given in Table 6-1. One further set of characteristics (Low HR) for bradycardic (< 60 bpm) subjects was obtained [364] to simulate low heart rate conditions. Heart rate was not stated for these subjects so 50 bpm, indicative of clinically significant bradycardia [364], was used.

Table 5-2 HRV characteristics determined from physiological data

Database characteristics	Low HR	Resting	Sleep	Meditation	Exercise
Number of subjects	25	20	16	12	20
Mean HR (bpm)	NS	62	72	78	111
Median RR-interval (ms)	NS	976	834	775	540
SDNN (ms) standard deviation, total variance	49	35	40	66	8.9
SDSD standard deviation of successive differences	34*	30	29	34	6.5

* RMSSD used in place of SDSD
Abbreviations: HR, heart rate; NS, not stated; RMSSD, sqrt(mean(diff(RR)^2)); SDNN, std(RR); SDSD = std(diff(RR)).

Two peak locations in the mid-range of the LF and HF frequencies were determined from the average of the physiological data: 0.07 and 0.24 Hz (Figure 5-2). These two frequencies already meet the requirement to minimise interactions from related harmonics.

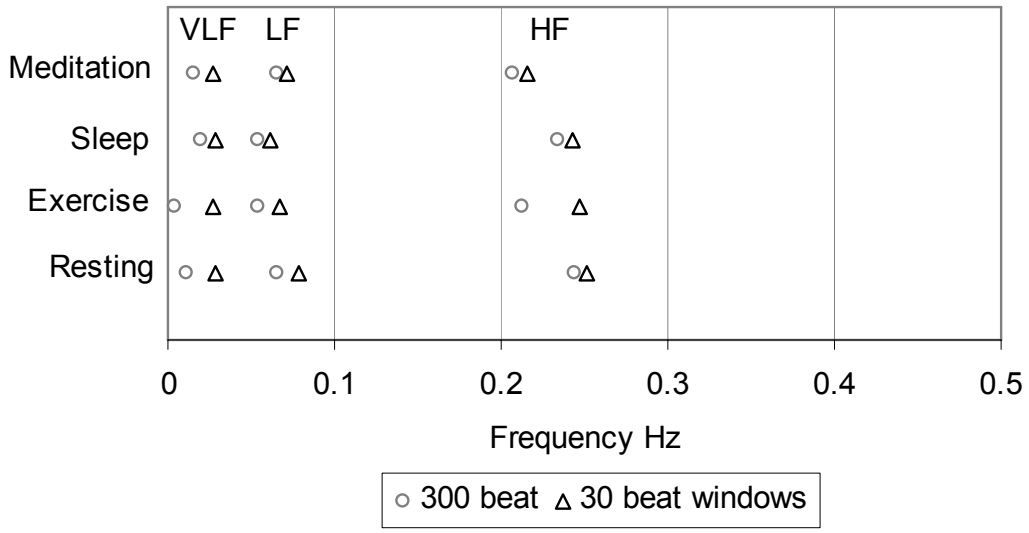


Figure 5-2 Location of frequency peaks in VLF, LF and HF for physiological data are at mean 0.028, 0.07 and 0.24 Hz respectively

The synthesised signal with the outer limit peaks of 0.07 and 0.24 Hz can be seen in Figure 5-3.

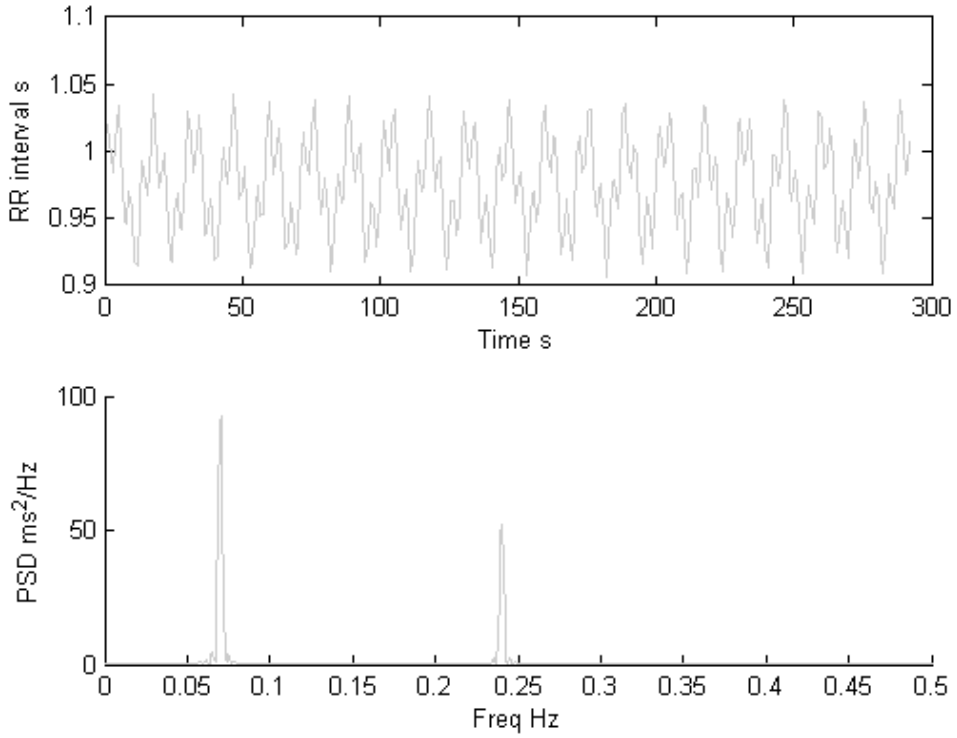


Figure 5-3 Synthesised signal with mid-range peaks at 0.07 and 0.24 Hz: 300 beats at mean RR-interval of 975 ms (top) and spectral power (bottom)

The parameters used for the synthesised realistic signals are listed in Table 5-3. The amplitudes, a_1 and a_2 were selected by trial and error to generate signals with the characteristic SDNN and SDSD.

Table 5-3 Sine wave parameters for synthetic signals

Database characteristics	*mRRI*	a_1	ω_1	a_2	ω_2
Bradycardia	1.205	0.068	0.07	0.02	0.24
Resting	0.976	0.039	0.07	0.029	0.24
Sleep	0.851	0.046	0.07	0.032	0.24
Meditation	0.775	0.085	0.07	0.035	0.24
Exercise	0.501	0.004	0.07	0.012	0.24

Abbreviations: a_1, a_2, amplitudes of the sine waves; *mRRI,* mean RR-interval; ω_1, ω_2, frequency peaks of the sine waves in Hz.

The mean and standard deviation of 1000 repetitions for window lengths from 600 beats down to 25 beats were determined for three sets of spectral power density measures: a) absolute power – LF, HF, TP and AP, b) percent change in power – LF, HF, TP and AP, and c) relative power – LFnu (LF/TP), HFnu (HF/TP) and LF/HF.

5.3.5 Noise

A linear trend similar to that which naturally occurs during the increased heart rate associated with exercise was added to the realistic synthesised HRV signals to assess if contamination to LF or HF power density occurred. The Exercise database was used to determine the size of the linear trend (Figure 5-4).

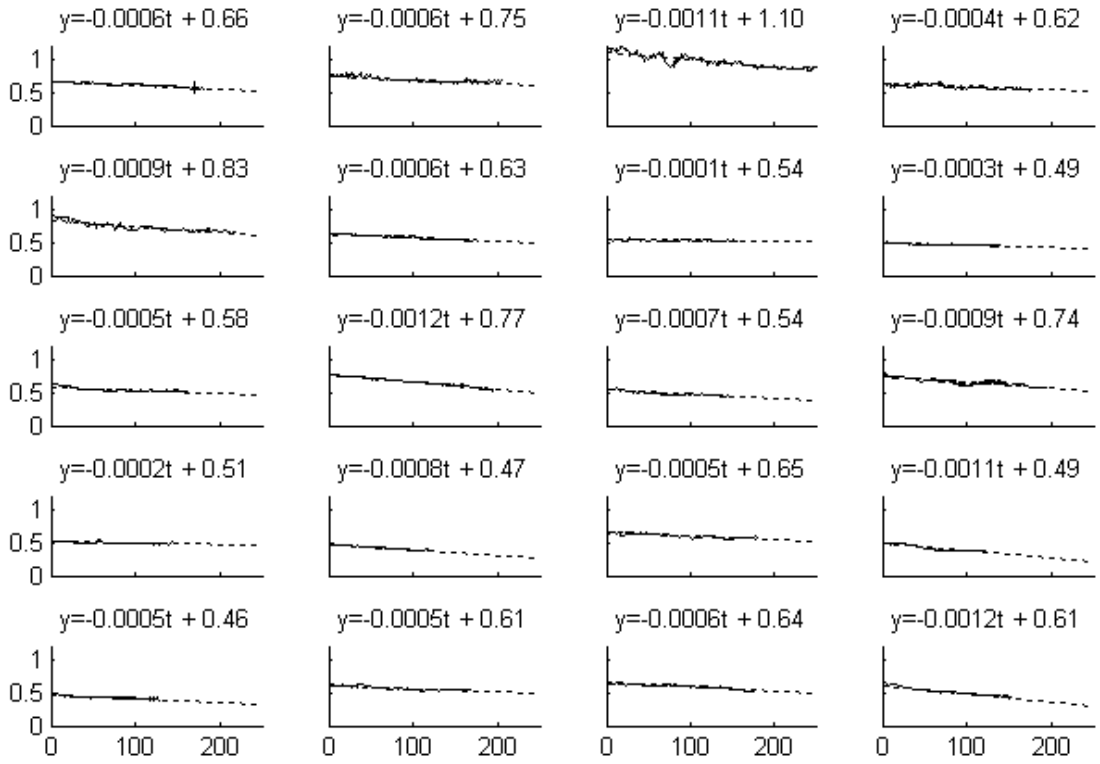


Figure 5-4 Linear trends for 16 subjects in the Exercise database for a 300 beat period, mean slope -0.00066t + 0.64

Two linear trends were determined. The larger of the two trends was the mean slope of the subjects in the Exercise database: -0.00066*t (with time in seconds). The trend can be seen as a large peak in the VLF region (DC to 0.04 Hz) of the spectral density plot in Figure 5-5.

Tests were also done with a smaller trend of -0.0001*t that may be more common in non-exercise situations (example in Figure 5-4, 3rd column, 2nd row).

The parameters previously selected to ensure SDNN and SDSD were similar to real data were used and were not adjusted for these tests with a trend.

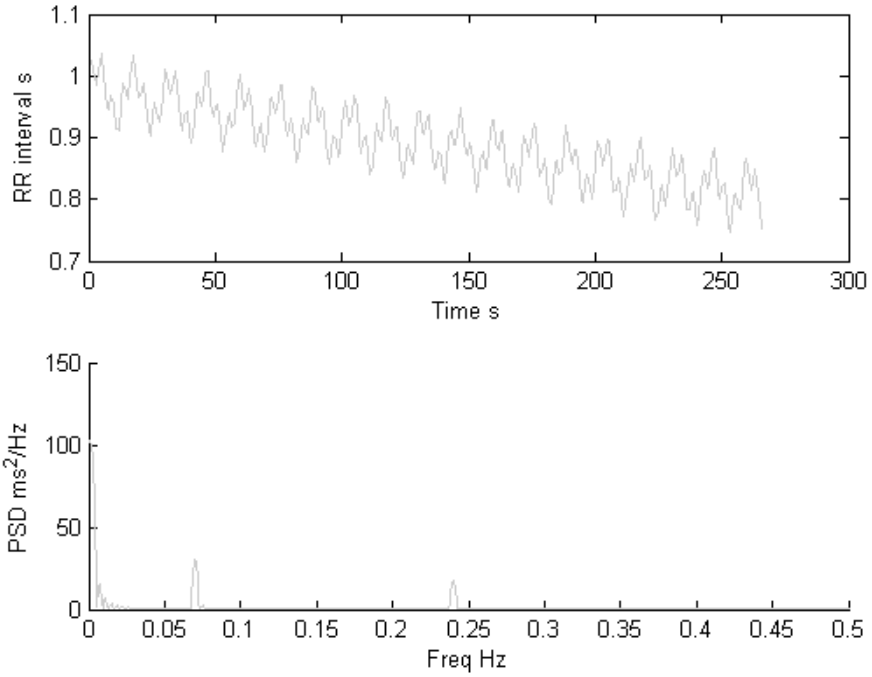


Figure 5-5 Synthesised signal with mid-range peaks and large trend: mean RR-interval of 975 ms (top) and spectral power (bottom)

5.4 Results

5.4.1 Resolution of outer limit peaks

The resolution of outer limit peaks (0.041 and 0.37 Hz) can be seen in Figure 5-6. On the left are the synthesised signals with a mean RR-interval of 975 ms for decreasing record lengths from 300 beats (top) to 25 beats (bottom). On the right are the resultant spectral density plots.

The spectral density plots also show four horizontal dash-dot lines that give the significance of the peaks from α = 0.001 (top line) to 0.05 (bottom line).

The outer frequency limits were able to be resolved with a significance of p = 0.05 for a 30-beat window with RR-interval of 975 ms, (Figure 5-6 c) but not with 27 or 25 beats (Figure 5-6 d and e).

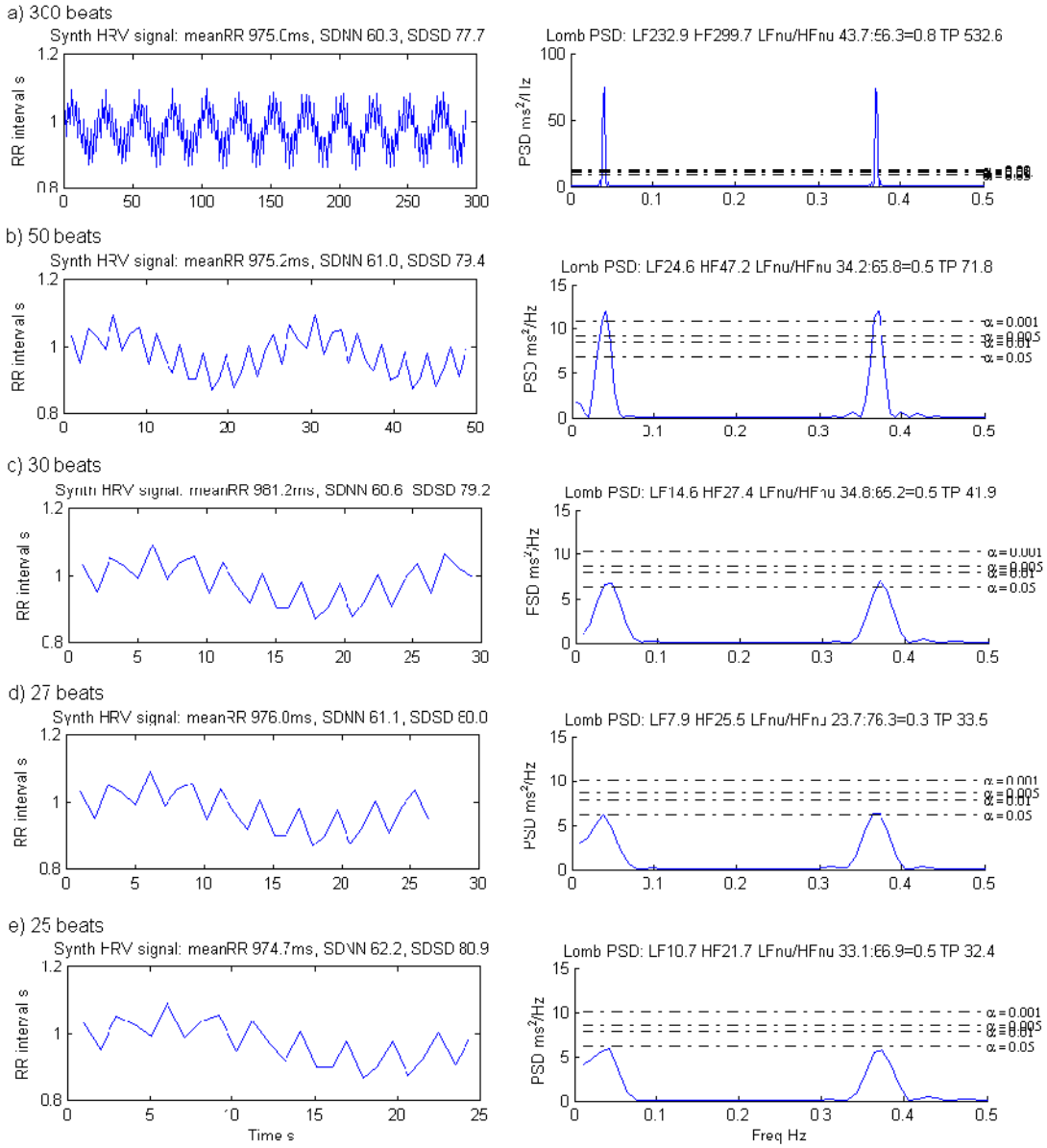


Figure 5-6 Outer limit (0.041 and 0.37 Hz) resolution for mean RR-interval 975 ms (Resting) Left: Synthesised signal with decreasing record lengths: 300, 50, 30, 27 and 25 beats (top to bottom). Right: Power spectral density with significance levels: 0.001, 0.005, 0.01 and 0.05 (top to bottom dash-dot lines).

5.4.2 Resolution of outer limit peaks for decreasing RR-intervals

With a mean RR-interval of 600 ms (Figure 5-7) both outer limits were able to be resolved down to a 30-beat window. At 500 ms typical of exercise (Figure 5-8), the LF peak was on the limit of detection (p = 0.05) with a 30-beat window.

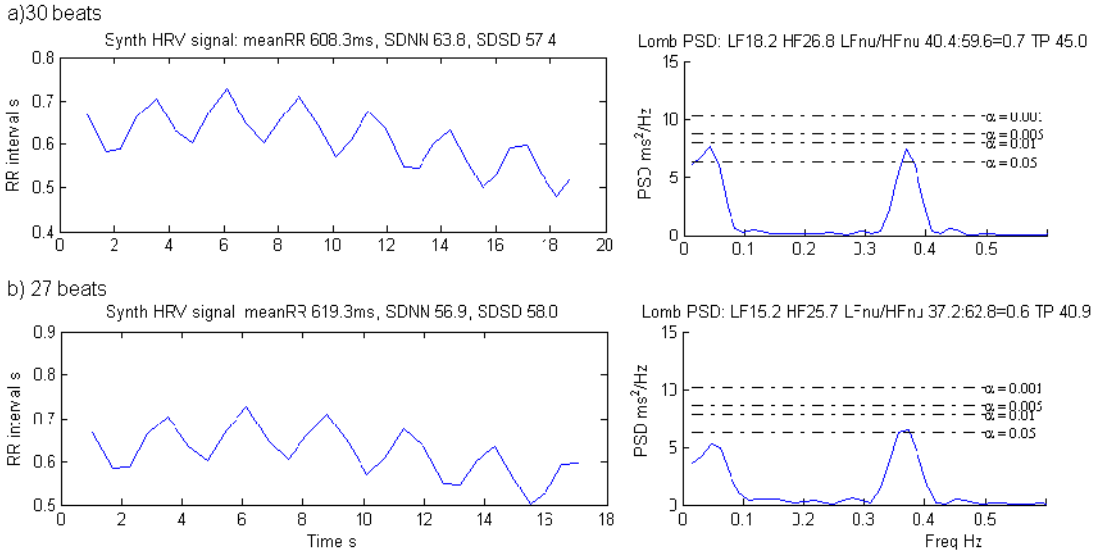


Figure 5-7 Outer limit resolution for mean RR-interval 600ms Left: Test signal for record lengths a) 30 and b) 27 beats. Right: Power spectral density

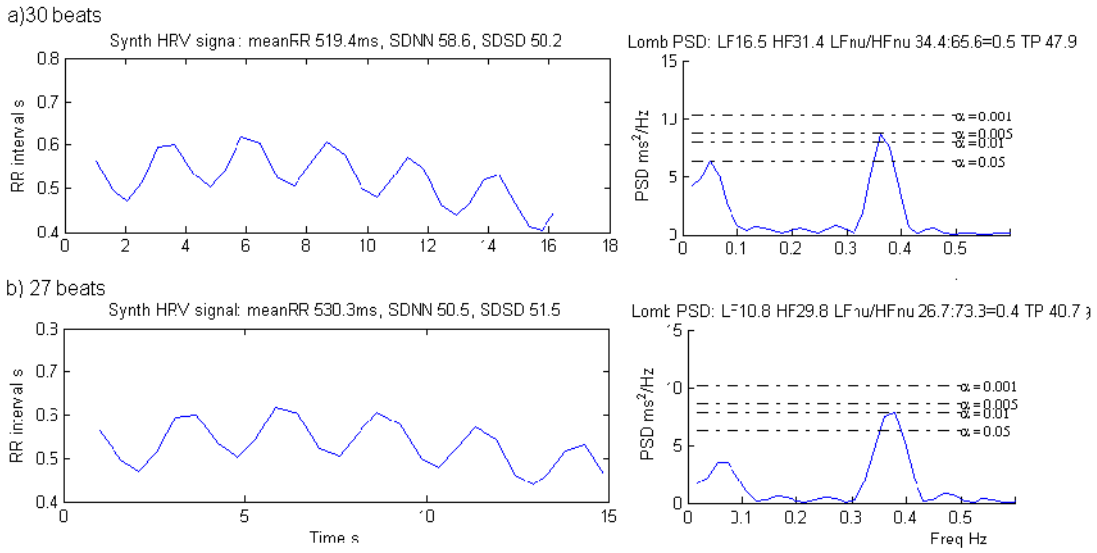


Figure 5-8 Outer limit resolution for mean RR-interval 500ms (exercise) Left: Test signal for record lengths a) 30 and b) 27 beats. Right: Power spectral density

5.4.3 Spectral power of outer limits

With outer limit peaks, the decrease in spectral power density for TP reflected the LF decrease for window sizes <600 beats and mean RR-intervals 975ms through to 500 ms (Figure 5-9). A small increase in LF power occurred at 30 beats with a mean RR-interval of 600ms.

HF showed little change until window size was <50 beats. For longer mean RR-intervals HF decreased, but for shorter ones (25 and 30 beats) conversely, HF increased.

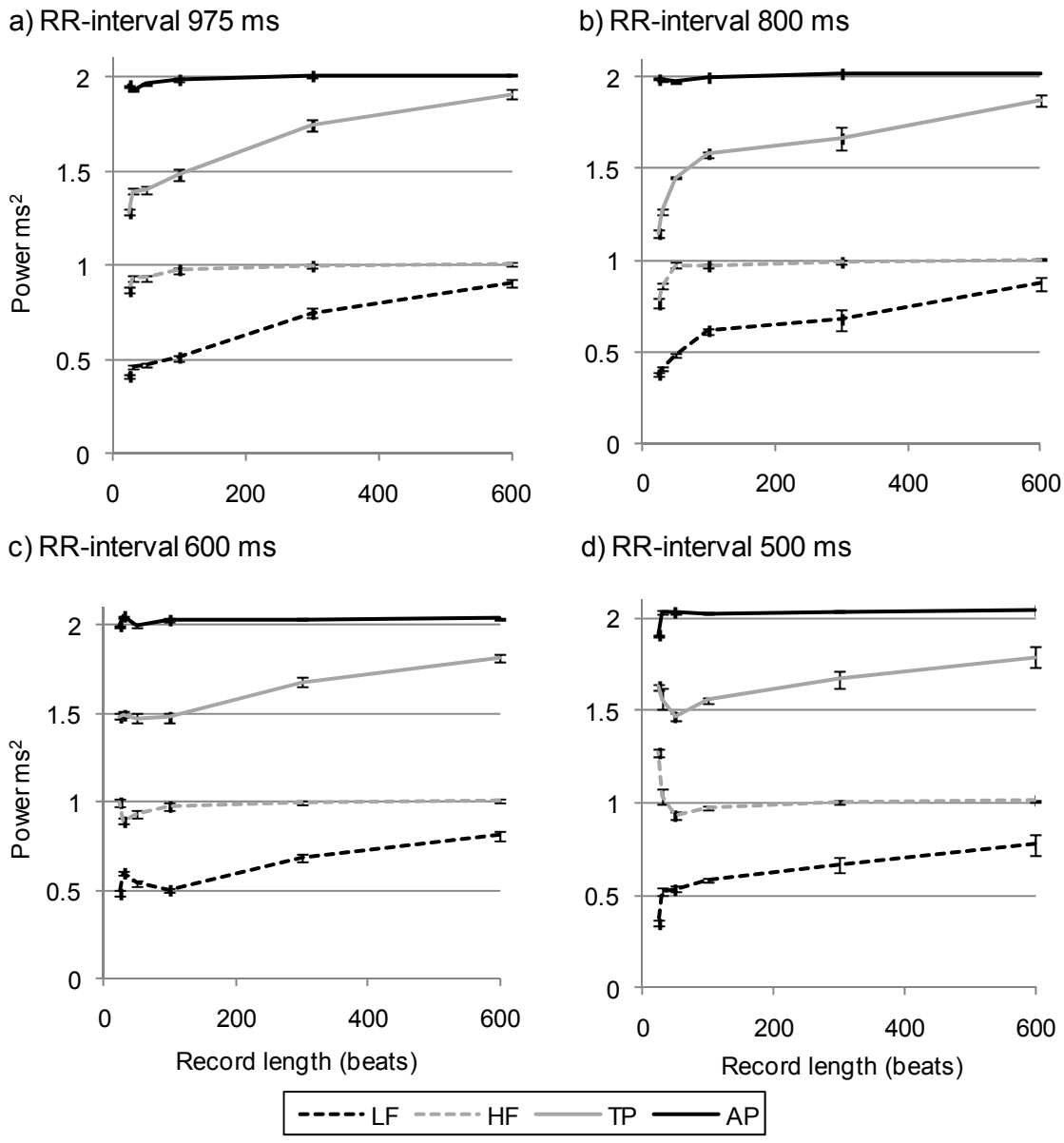


Figure 5-9 Power of outer limits (0.041 and 0.37 Hz) mean RR-interval a) 975 ms, b) 800 ms, c) 600 ms, and d) 500 ms, error bars show standard deviation

5.4.4 Validation with realistic synthesised data

Synthesised realistic signals

The synthesised signals have visible characteristics of real RR-interval time series (Figure 5-10). For each physiological state, real data from 3 to 5 subjects (in different colours) is shown for 300 beats (top) and zoomed to 50 beats (middle) for comparison to 50 beats of synthesised data (bottom). RR-interval data for bradycardia subjects was not available.

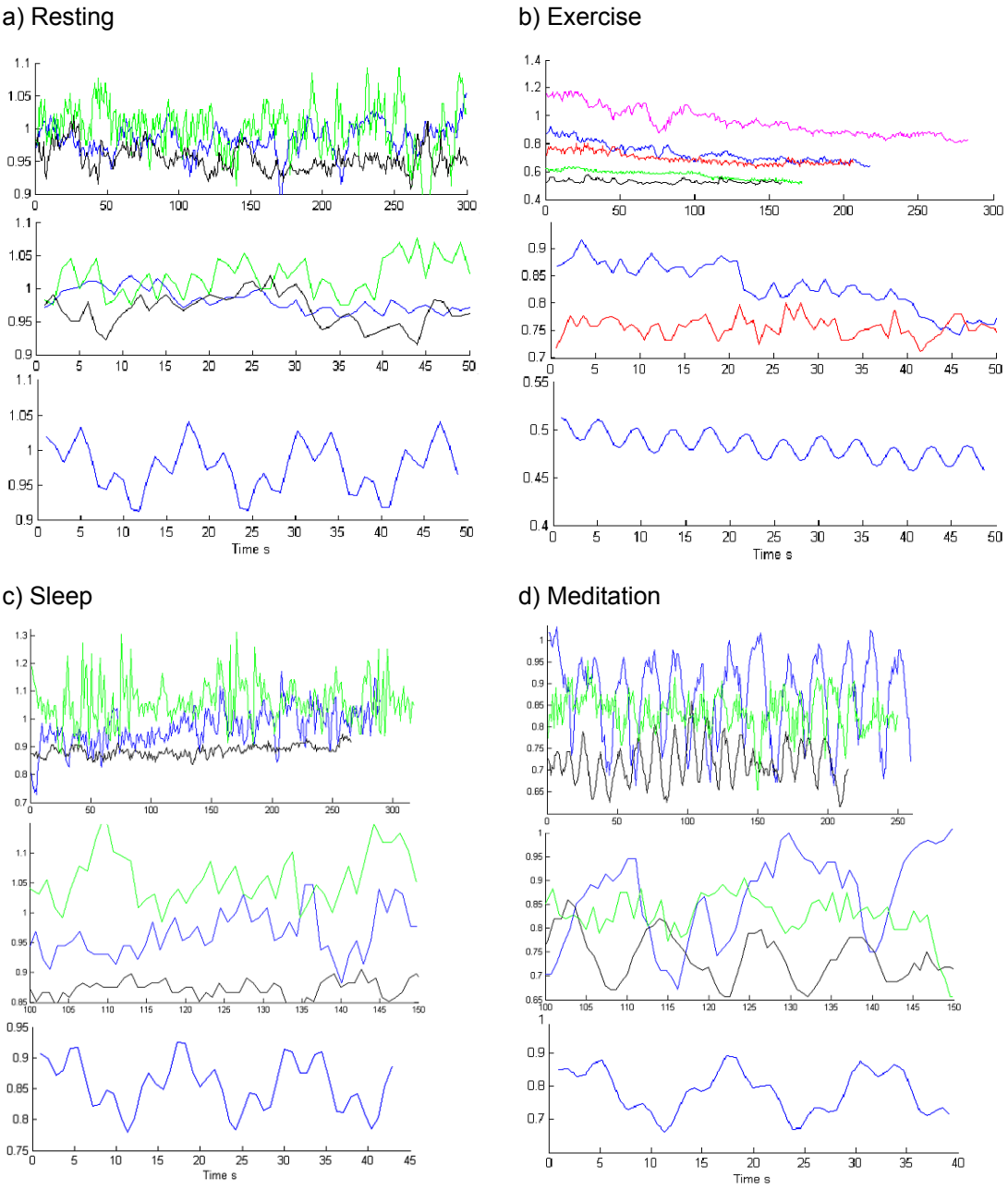


Figure 5-10 Mean RR-intervals from PhysioNet databases for 3-5 subjects (colours), 300 (top) and 50 beats (middle) compared to 50 beats synthesised data (bottom) for a) resting, b) exercise, c) sleep, and d) meditation.

Absolute power

With a more realistic synthesised signal (mid-range peaks) the absolute power of spectral peaks decreased slightly with shorter record lengths (Figure 5-11):

- AP, TP and LF power decreased similarly, TP change reflecting the larger component of LF, except in exercise where HF was the larger component.
- For meditation, HF increased slightly with record lengths <50 beats, but this was only a small component of TP.
- The difference between AP and TP increased at short record lengths indicating increased VLF power.

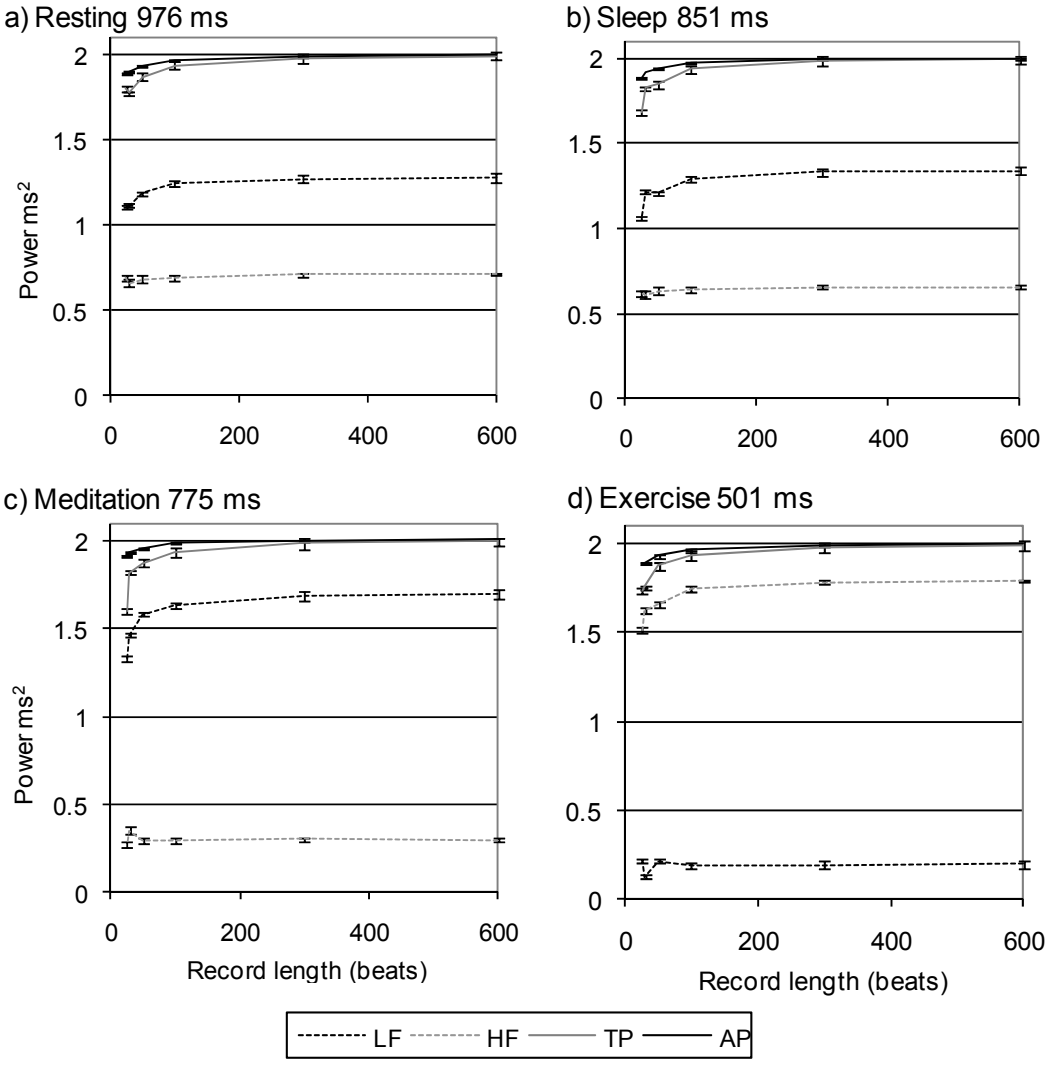


Figure 5-11 Absolute power for mid-range peaks, mean RR-interval a) 976, b) 851, c) 775, and d) 501 ms error bars show standard deviation

Percent change in power

The amount of change in the absolute power is more clearly seen in plots of percent power change, Figure 5-12. There was a 5% decrease in AP spectral power density and a 10% decrease in TP for 30 beat records.

With shorter RR-intervals and shorter record lengths, large changes were seen in HF for meditation, and LF in exercise, but these have only a small impact as they were only a small component of total power (visible in Figure 5-11).

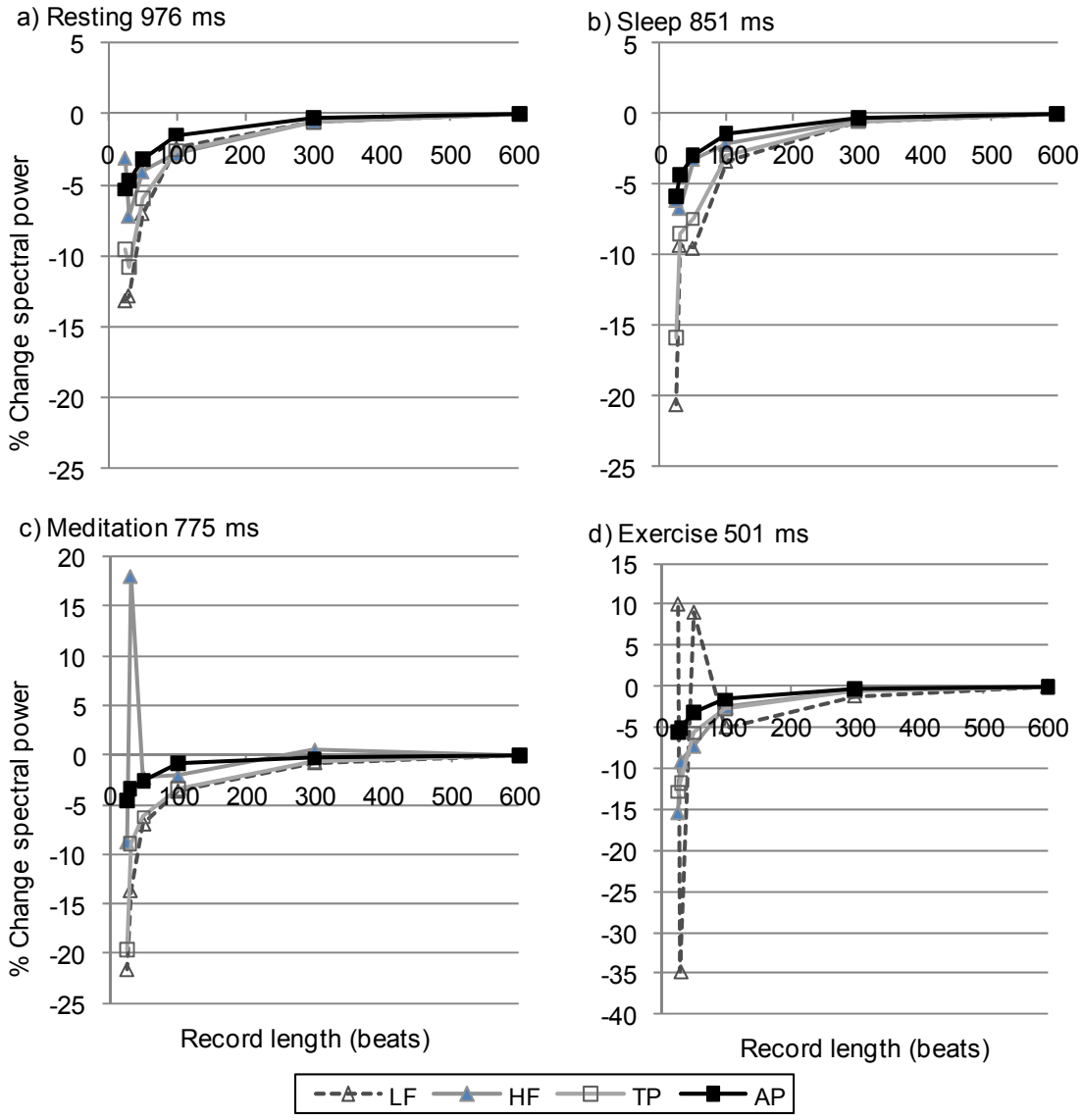


Figure 5-12 Percent change in power of mid-range peaks mean RR-interval a) resting 976 ms, b) sleep 851 ms, c) meditation 775 ms, and d) exercise 501 ms Note: y-axis scales are different, record lengths: 25, 30, 50, 100, 300 and 600 beats

Relative power

The relative spectral measures in normalised units (Figure 5-13) show a small change from 600 to 30 beats: LFnu 2.3%, 0.9%, 5.2% (for Resting, Sleep and Meditation respectively), and HFnu 2.9% for Exercise.

The exceptions are at shorter record lengths: a) LF/HF in meditation decreased however, its value was still far higher for meditation than any of the other synthesised physiological states; and b) HFnu in exercise increased and decreased, but again the value of HFnu in exercise was much higher than in any of the other physiological states.

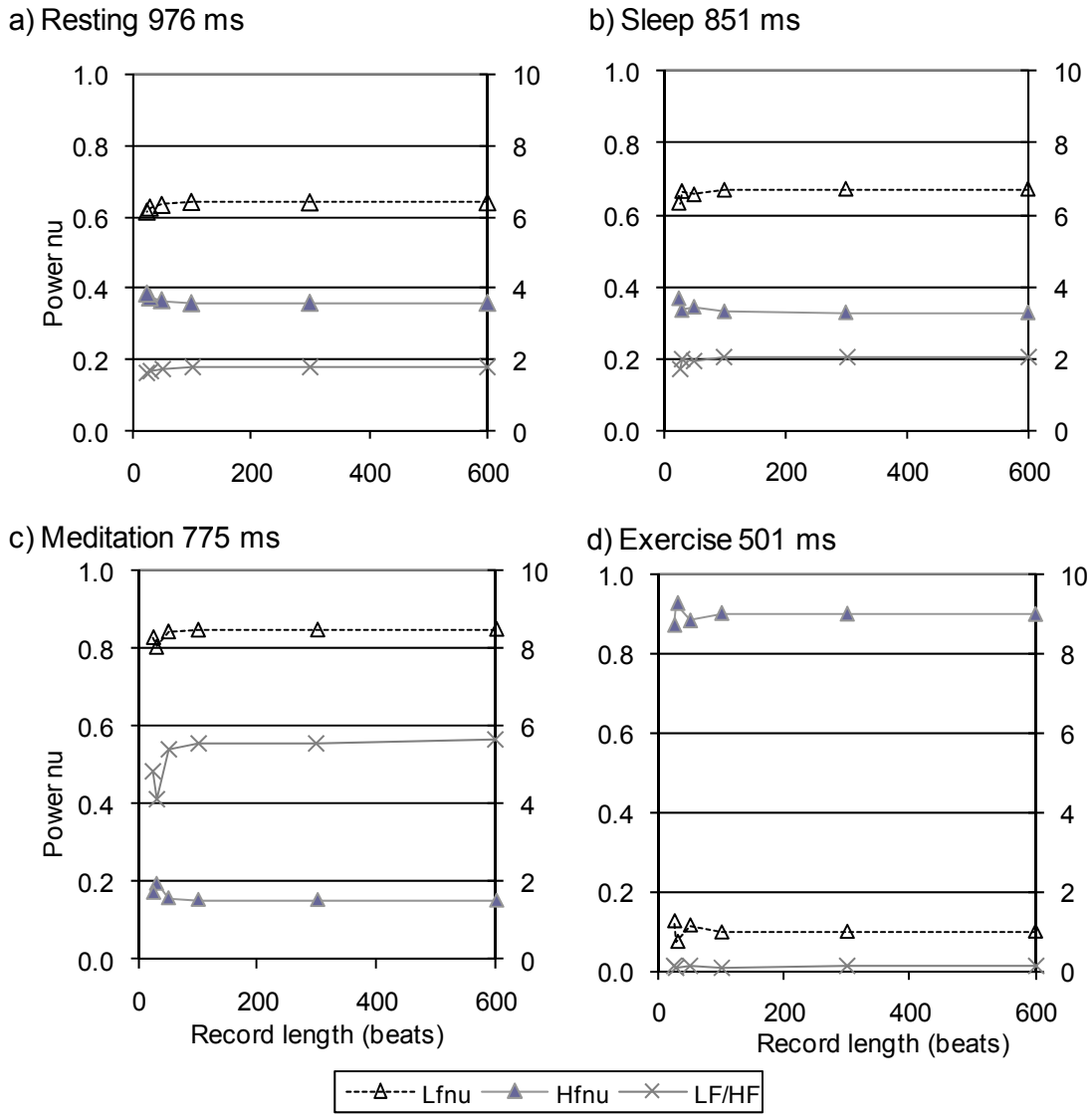


Figure 5-13 Relative power, LFnu, HFnu, and ratio LF/HF (right axis) of mid-range peaks with decreasing window length for mean RR-interval a) resting 976 ms, b) sleep 851 ms, c) meditation 775 ms, and d) exercise 501 ms.

Bradycardia

With a low heart rate of 50 bpm, the absolute power showed a small decrease similar to other physiological states (Figure 5-14 a).

The relative spectral measures (Figure 5-14 b) only showed a decrease in LF/HF similar to meditation but again the LF/HF component was much larger than for other physiological states, even meditation.

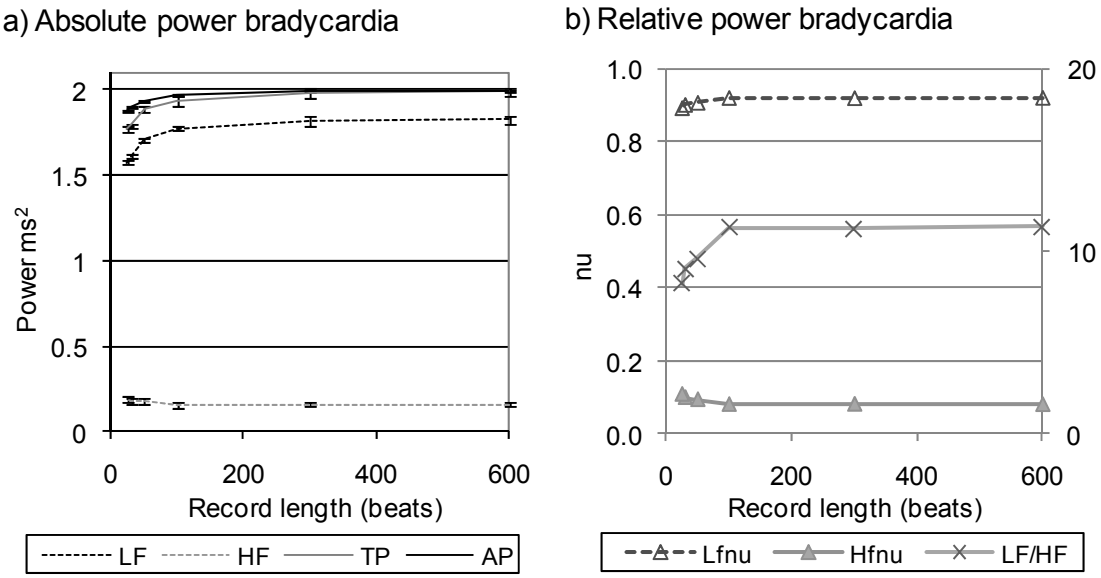


Figure 5-14 Power for bradycardia, mean RR-interval 1200 ms a) relative (error bars show standard deviation) and b) absolute power of mid-range peaks

5.4.5 Addition of linear trend

Small linear trend

The plots for a small negative linear trend of 0.0001t (Figure 5-15) show absolute power on the right and relative power on the left for a) sleep, and b) exercise.

In long record lengths the absolute power in LF, HF and TP was decreased, particularly at short RR-intervals (i.e. exercise). The difference between AP and TP showed most power was in the VLF range. As record length decreased less power was in the VLF range, and power in LF, HF and TP approached that with no linear trend (compare with Figure 5-11). The relative power measures remained the same as with no linear trend for exercise and sleep (compare with Figure 5-13).

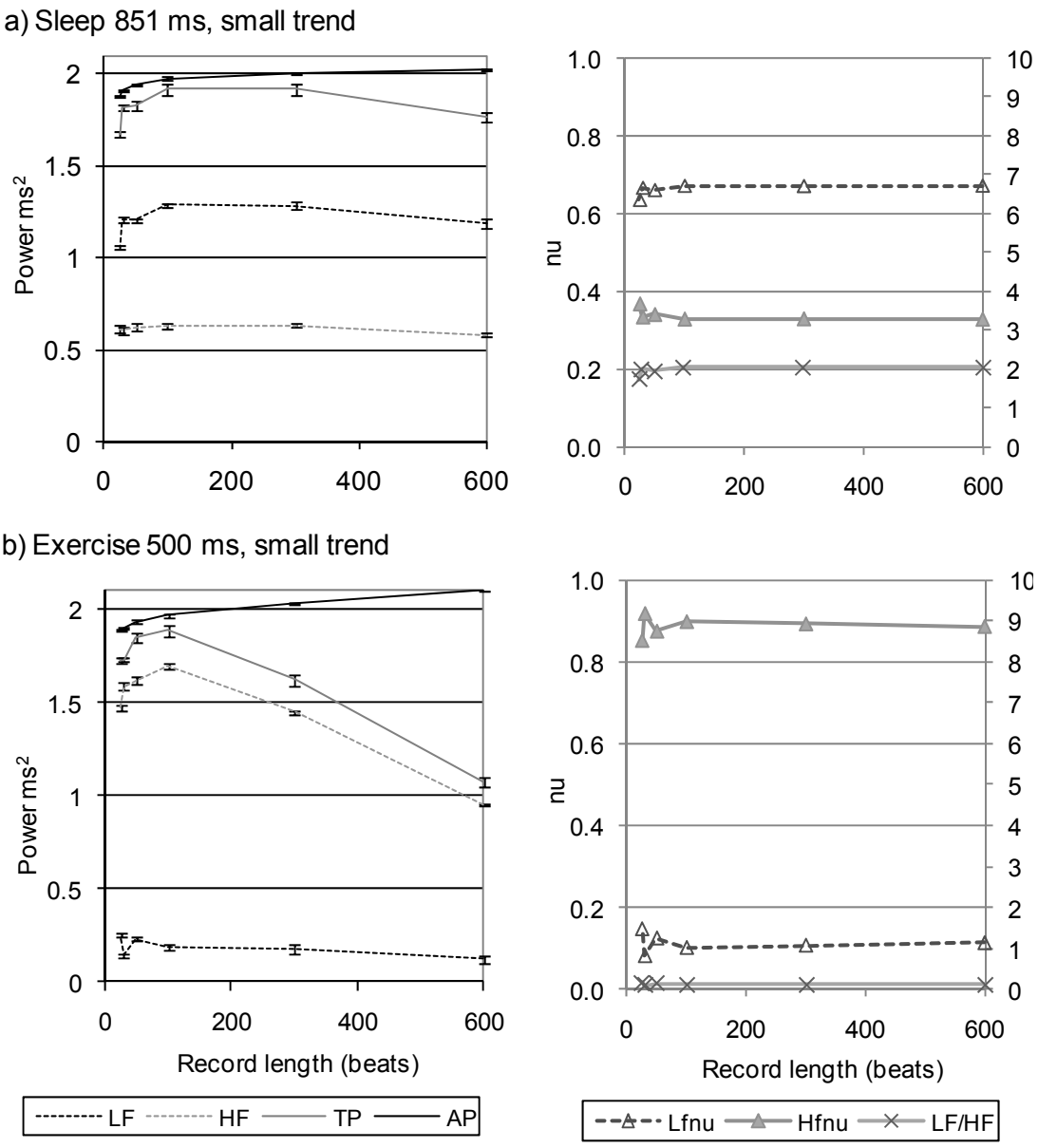


Figure 5-15 Addition of small linear trend, 0.0001t, VLF noise: absolute spectral power (left) and relative power (right), mean RR-interval a) sleep 851 ms, and b) exercise 501 ms

Large linear trend

Plots for the larger negative trend, 0.00066t, similar to exercise, are shown for a) resting, and b) exercise (Figure 5-16). The absolute power in LF, HF and TP is substantially decreased for longer records. With shorter record length, absolute power increased approaching values with no linear trend (compare with Figure 5-11).

The large trend caused a decrease in relative power for longer record lengths and short RR-interval particularly in exercise (compare with Figure 5-13). At

shorter record lengths, relative power for resting approached those with no trend, but for synthesised exercise showed HFnu lower, and LFnu higher than expected.

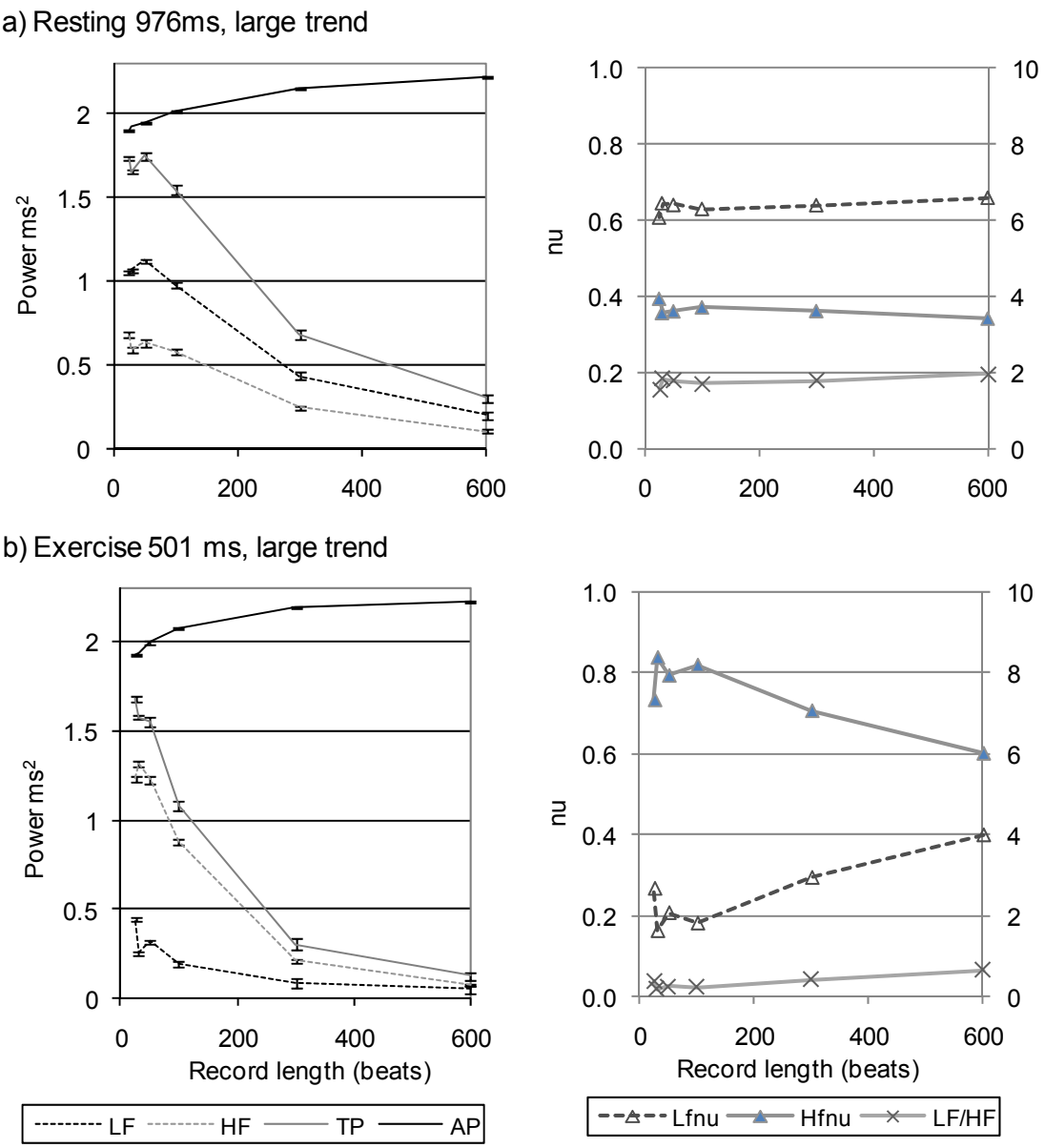


Figure 5-16 Addition of large linear trend, 0.00066t, similar to exercise absolute spectral power (left) and relative power (right) mean RR-interval a) resting 976 ms, and b) exercise 501 ms

5.5 Discussion

5.5.1 Resolution of LF and HF outer limits

With a 30-beat window the outer limit peaks of 0.04 and 0.4 Hz were able to be resolved for RR-intervals from 975 ms down to 500 ms typical of exercise using the Lomb-Scargle algorithm. This is a window length of 30 s to 15 s.

The spectral power for these atypical signals in windows <600 beats show a decrease in LF that was reflected in TP. This is due to the power spectral density peaks getting wider as the window lengths decrease, with some of the power extending below the LF region (0.04 Hz) and above the HF region (0.4 Hz). For the peak frequencies that were selected to test the outer limits, 0.041 and 0.37, they were not equidistant from the edge of the measurement area; the lower peak is closer to the LF limit therefore is more affected by the widening peak decreasing the area above 0.04 Hz that is measured.

5.5.2 Outer limit HF contamination with high heart rate

With the outer limit peaks, HF power remained constant down to 50 beat records then decreased for longer RR-intervals, and increased for shorter RR-intervals. This may indicate HF contamination in short records and with high heart rate conditions. Figure 5-8 showed that rather than HF contamination, the cause is less LF data being measured by the 30-beat window.

5.5.3 Synthesised realistic signals

A simple sine wave function was used to create two peaks (or three with trend) for the synthesised signals with characteristics taken from real data: mean RR-interval, SDNN, SDSD, peak frequency locations for LF and HF ranges, and linear trend. The synthesised signals were visually shown to have patterns characteristic of the real RR-intervals from the PhysioNet databases. A random component was not included to ensure the source of variability was limited to resolution by the Lomb-Scargle algorithm.

With realistic synthesised signals, the absolute power decreased for shorter record lengths. The 10% decrease in TP was due to the larger component, usually LF, but HF in exercise. The change to relative power was minimal,

2.9% on average from 600 down to 30-beat record lengths; the exception at short record lengths was a 20% change in LF/HF in both meditation and bradycardia however, the magnitude of LF/HF is much larger for these states and still allows differentiation from each other and from the other physiological states.

5.5.4 LF limits

It is expected that worse case conditions for measuring the LF spectral power will be in short windows with conditions of higher heart rate typical of exercise, where the lower RR-interval of 500 ms is equivalent to a heart rate of 120 bpm. Under these conditions, a 30-beat window is only 17-19 s, however, with these realistic synthesised signals, LF is only a small component of the total power, so there is little impact on the absolute or relative power measures.

This study was limited by the mean heart rate of 120 bpm available in the physiological databases used to create the SDNN and SDSD characteristics for the simulated HRV signal. These LF worst case conditions need to be further tested for exercise data with a higher mean heart rate.

Although the Lomb-Scargle technique has been widely used in other fields for its ability to be used with incomplete data series and large data gaps (e.g. missing seasonal data in geophysical time series [365]) it has not been mathematically validated for use with only partial low frequency cycles that occur in short-term HRV analysis.

5.5.5 Low heart rate

Bradycardia of 50 bpm and with characteristics (SDNN and SDSD) typical of a real population did not introduce high frequency contamination reported by Laguna *et al*. [165]. They identified high frequency contamination for the extreme low heart rate conditions occurring in abnormal atrioventricular conduction where a subjects heart rate drops (e.g. for 2:1 block, heart rate drops to half the normal heart rate, about 35 bpm for the subject they investigated). This unusual condition also has a signal with sharp changes between normal heart rate and the low heart rate regions.

5.5.6 Contamination by high-frequency components

High frequency non-stationarities have been shown by Laguna *et al.* [165] to cause high frequency contamination.

The highest HF component in the realistic synthesised signals used here was in meditation (parameter a_2 in Table 5-3). A large percent change in HF power was seen for meditation, but this was only a small component of the overall power of the realistic synthesised signal so had no statistically significant effect.

Further tests inserting a higher HF component (similar to the VLF component trend) are needed if high frequency non-stationarities are expected. Data with obvious large high frequency non-stationarities (such as the atrioventricular block condition investigated by Laguna *et al.*) are usually excluded from analysis and were not in the physiological data used in this study. This HF component may be modelled by a step function causing a baseline shift, rather than by addition of a third frequency peak.

5.5.7 Nonstationarity: trend or VLF noise

Longer record lengths were substantially affected by the addition of a trend with decreased absolute power, and for short RR-interval (exercise) decreased relative power.

For shorter record lengths, < 50 beats, both absolute and relative powers were largely unchanged and approached values with no trend.

Non-stationarities in the form of VLF noise (linear trend), although having a large effect on >100 beat windows, had little effect on 30-beat windows for relative or absolute power.

5.6 Summary

Relative spectral power analysis (LFnu, HFnu and LF/HF) over 30-beat windows using a Lomb-Scargle algorithm is able to provide the same data as over 300 beat windows with: a) the minimal noise present in synthesised

realistic HRV signals, and b) linear trends (VLF noise) without the detrending required by longer windows.

Although the minimum recommended time for analysing LF spectral power is 2 min, the Lomb-Scargle analysis showed minimal change (2.9%) in relative power from 600 beats down to short 30-beat windows even for RR-intervals down to 500 ms typical of exercise, a window of only 17-19 s.

The absolute spectral powers, LF, HF and TP, at 30-beat windows decreased by about 10% from the longer 600 or 300 beat windows, but decreased consistently for the range of RR-intervals characteristic of physiological states.

The addition of a negative linear trend (VLF noise) has minimal effect on relative or absolute powers with short 30-beat records.

The spectral indices based on the Lomb-Scargle algorithm will be validated with real data in the next Chapter to determine if they can be useful in differentiating between physiological states with only 30-beat windows.

Chapter 6. Validation of very short-term indices

6.1 Aim

To monitor the dynamic changes in this study, very short-term HRV analysis (< 60 s) is needed. The HRV indices that may be useful for short-term analysis have been identified (Chapter 4), and the minimum window that still retains relevant spectral power has been determined to be 30-beats (Chapter 5).

Before assessment can be undertaken of the dynamic activities with fentanyl, the identified short-term HRV indices, including spectral indices, need to be tested with sympathetic and parasympathetic activities. This is achieved using real data in different physiological states: resting, active, exercise, sleep, and meditation.

A minimum set of indices is identified for future use by a) removing redundant indices that are highly correlated with the commonly used time and spectral domain indices and selecting only one of indices highly correlated with each, b) establishing confidence intervals for the minimum set of indices with real data over a 30-beat window in resting and other physiological states, c) comparing very short-term results to the longer periods in the literature, and d) removing indices that were not able to differentiate the real physiological databases.

6.2 Background

6.2.1 Correlations between indices

Many indices calculated over periods of 2 min to 3 hr are correlated with the traditional time domain indices, SDNN and RMSSD [152], which are recognised as surrogates for many other indices that are difficult to measure, including frequency domain indices [179, 318].

Indices that do not correlate strongly with SDNN or RMSSD over a variety of physiological states may prove useful in determining dynamic characteristics of cardiac nervous system activity.

6.2.2 Limited use

Many of the lesser known indices have had very limited use in only one or a few small specific studies, and often only by the creator of the index, for example, assym(R/L) for neonatal sepsis [323], pQa-c sleep stage in cats [202], r(RR) sleep in men [205], and many have only been used for differentiating a cardiac disease state: acv0x and mean.r(L1-6) coronary heart disease [326], CV atrial fibrillation [349], gradRR myocardial infarction [186] kurtRRz myocardial infarction [354], PolVar20 cardiomyopathy [359], TACI congestive heart failure and atrial fibrillation [342].

These indices are worthy of wider investigation, across a range of physiological states, to determine a) if they are correlated to other indices and b) their usefulness to general HRV applications.

6.3 Method

6.3.1 Databases

The effectiveness of very short-term indices to detect parasympathetic and sympathetic activity was tested on databases of subjects covering a range of physiological states that could be expected to have differing activity levels of the autonomic nervous system ranging from resting and sleep (high parasympathetic activity) to exercise (high sympathetic activity) and meditation (mixed activity). Five PhysioNet databases [363] were used (summary in Table 6-1).

Twenty subjects were used in Resting, Active and Exercise databases. For the Sleep data, only one of the sleep databases archived in PhysioNet had annotations for sleep stage, apnoeas and arousals limiting the subjects to 16. Meditation was limited to 12 subjects from the only trial archived.

For each subject, a section of 300 beats was taken 5 min into the start of the record, except for a) the Exercise group where the section was taken during the steady decline in RR-intervals usually 10-15 min into the record, and b) the Sleep group where the selection was the first block of 300 beats in stage 2 sleep with minimal apnoea or arousal.

The Resting subjects were supine for 2 hr in a wakeful rest state while watching a movie (Fantasia) to stay awake [366]. They were all healthy with no known cardiac disease.

Table 6-1 Descriptions of PhysioNet databases

Group	Database	N	f_S Hz	F	M	Age mean (range)
A.Resting	Fantasia database (fantasia) [366], no arrhythmias, subjects resting supine while watching movie to remain awake	20	250	10	10	50 (21-77)
B.Active	MIT-BIH normal sinus rhythm (nsrdb) and Normal sinus rhythm (nsr2db), no arrhythmias, 24 hr Holter	20	128	13	7	38 (20-67)
C.Exercise	MIT-BIH ST change database (stdb) [367], transient ST changes during exercise	20	360	NA	NA	NA
D.Sleep	MIT-BIH Polysomnographic database (slpdb) [363], with sleep stage annotated	16	250	0	16	45 (32-63)
E.Meditation	HR oscillation during meditation [368] 1 hr	C 8 Y 4	128	7	5	NA (C 29-33) (Y 20-52)

Abbreviations: C, Chi meditation; F, female; f_S, sampling frequency; HR, heart rate; M, male; MIT-BIH, Massachusetts Institute of Technology and Beth Israel Hospital; N, number of subjects; NA age and gender not provided; Y, yoga mediatation.

The Active subjects had no significant arrhythmias (12 hr recordings available). The data selection avoided areas where heart rate was increasing in a steady manner indicative of light exercise, or where sudden large changes in heart rate occurred.

The selected sections were moved slightly if necessary to avoid visually identified missing or extra beats. If supplied, the annotations within the PhysioNet databases were used to confirm normal sinus rhythm for selected beats.

6.3.2 Data analysis

Indices were calculated over ten consecutive, non-overlapping, 30-beat windows. This covered a period of about 5 min for each subject that enabled comparison with published results. This window size of 30-beats was the

smallest that was still useful for analysing the Lomb power spectrum density above 0.04 Hz (Chapter 5). All data analysis was performed with MatLab software (The MathWorks Inc., Natick, MA, USA).

6.3.3 Correlation between indices

The correlation between indices was tested over very short 30-beat windows for all subjects in the five physiological states. Indices correlated with commonly used time and spectral domain indices were excluded from further analysis. Where indices were correlated with other indices, one was selected and the rest excluded. To test for a strong correlation between indices, the threshold was set to $r \geq 0.9$ ($p<0.001$). The average for each subject, rather than multiple data points, provided independent data points [369].

Normality of the data was tested to determine the correlation statistic that should be used: Pearson for normally distributed data or Spearman (rank) for non-normal distributions.

The normal distribution was tested with either the Shapiro-Wilk or Shapiro-Francia test (null hypothesis that a sample comes from a normally distributed population) for sample sizes between 3 and 5000 [370]. Shapiro-Wilk is preferred for platykurtic, and Shapiro-Francia for leptokurtic samples (kurtosis <0 and >0 respectively) [370].

In conjunction with removing correlated indices to minimise the likelihood of false positives, the number of indices was further reduced by removing indices that had high rates of zeros or high rates of undefined numerical results.

6.3.4 Correlation with mean heart rate

Mean heart rate is a major determinant of HRV and must be taken into consideration when calculating HRV [274, 282, 344]. The selected indices were tested for correlation with mean heart rate to determine if further correction for heart rate should be made.

6.3.5 Stationarity

Analysis of HRV with spectral analysis requires stationarity of the signal for the output to be meaningful. Slow linear trends cause an increase in the LF

component ([371, 372] and frequent sudden changes in heart rate create wide band noise [373].

The weak stationarity required by spectral analysis can be verified by monitoring the mean and variance of the signal over the period of interest. This can be tested in a variety of ways:

- Visual inspection; requires experience and may be subjective [374, 375].
- Autocorrelation decay time [376].
- Construction of pole diagram [153].
- Kolmogorov-Smirnov empirical cumulative distribution function; can be sensitive to outliers [377].
- Reverse arrangements [378, 379]; with the assumption (that may be unrealistic for HRV data) that the nonstationarity of the data will be revealed by time trends.
- Variance in subset means and variances [153, 380].
- Stationarity statistic, StatAv; variance of subset means divided by overall variance [381], or mean corrected, StatAvc [382], if stationarity is related to the mean RR-interval.

The last of these, StatAv, was used in this study because of its normalisation to overall variability. The data were tested for stationarity over 300 and 30 beat segments with division into ten (30 beat) and five (6 beat) subsets respectively. The standard deviations of the means for each subset were divided by the overall standard deviation. The average and range for 10 of the 5x6 beat subsets was used to cover the same period as the 300 beat window.

6.3.6 Confidence intervals

Confidence intervals of the uncorrelated indices were established for the 5 databases. Bootstrapped[6] 95% confidence intervals of the median [225, 383,

[6] Bootstrapping is a statistical method for estimating the sampling distribution of an estimator by repeated sampling with replacement from the original sample

384] were analysed in MatLab with 10,000 replications required [385]. The median is considered a more valid definition of centre with skewed frequency distributions because its accuracy is less influenced by variations in distributional shape [386]. The bias-corrected and accelerated percentile method was used for its qualities: transform respecting and second-order accuracy [387] making it best for dealing with skew and bias in the bootstrap sampling distribution [386, 388]. The bias-corrected and accelerated method can fail when the jackknife[7] is performed on non smooth data (e.g. median) and returns undefined results [387]. For these indices (indicated in this paper by ^ after the index name), the corrected percentile method was used [389].

6.3.7 Non-useful indices

Indices that were not useful in discriminating any of the different physiological states from Resting were removed.

6.3.8 Statistical considerations and significance

Redundancy

Redundant indices for removal were defined where the averages of each index over the baseline for each subject were highly correlated (Pearson or Spearman's rank correlation coefficient, $r>0.90$, $p<0.001$) over the range of the different physiological states.

Significance of differences between databases

The null hypotheses for this study are that each index does not differentiate between Resting and the other physiological states. The alternative hypotheses are that a difference can be shown.

Overlap of 95% confidence intervals was used to identify indices showing significant differences between databases: $p<0.006$ abutting, not overlapping confidence intervals, $p<0.04$ with overlap of one arm <50% (or <1/4 total confidence interval width). This has been shown to be acceptable for samples of ten or more, and with a difference in the confidence interval arm width of less than two [390, 391].

[7] The jackknife is a similar estimation method to bootstrapping but systematically recalculates the statistic estimate leaving out one observation at a time from the sample set.

Sample size and effect size

Cohen provides guidelines for the effect size of the difference between independent means divided by the standard deviation for small, medium and large effect sizes of 0.2, 0.5 and 0.8 respectively [392].

For this study with the indices measured over very short-term 30-beat intervals, the size of the difference between medians and the standard deviation of the databases is unknown, thus the effect size is not determined.

In general with a power of 80% and alpha = 0.05, Cohen advises a minimum of 26, 64 and 329 subjects respectively for large (0.8), medium (0.5) and small (0.2) effect sizes respectively. This study will not reach a power level of 80%.

Correction for multiple tests

The use of multiple tests of the difference between physiological states for each of the indices requires a correction factor to keep Type I (false negative, not recognising a true effect as significant) and Type II (false positive) errors to a minimum.

The conservative, but commonly used, Bonferroni correction adjusts table-wide Type-I error for all tests by using a corrected alpha (divided by the number of tests) for determining the level at which null hypotheses can be rejected.

There have been improvements to the Bonferroni procedures (e.g. Holm's sequential Bonferroni procedure where the number of tests in the denominator is reduced by the rank [393]) but they still have a number of disadvantages: they control the probability of false positives at the cost of increasing the probability of false negatives thus reducing power; and the more tests performed, the lower the probability of finding a result. This is a particular problem for studies like this one, with detailed analysis (more variables) that requires multiple statistical tests with small numbers of replicates and high variability. The subsequent low statistical power of Bonferroni procedures decreases the probability of finding a significant result [393, 394].

A less strict method recommended for exploratory analysis is the false discovery rate, FDR, developed by Benjamin and Hochberg [395, 396], that weights the Bonferroni correction by the rank of the p-value. While Bonferroni keeps the chance of Type I errors low at the expense of Type II errors, the FDR allows a proportion of false positives among all significant tests which can be identified in follow-up confirmatory studies with more power [396]. FDR is applicable to tests that are independent and also to those that are positively correlated [397, 398] and has been selected for use in this study.

With confidence intervals of the median for each database (non-normal distributions), the exact p-values have not been obtained for the comparisons between physiological states; however, the proportion of overlap, POL, of the confidence intervals is related to the p-values. The POLs are ranked with specific p-values identified (p = 0.2, 0.05, 0.01 and 0.001 for POL = 1, 0.59, 0.14 and -0.37) [390].

By plotting the ranked POLs for each test, against the rank-corrected alpha, they can be tested for the point where the p-value < corrected alpha (Figure 6-1). For these tests, with α = 0.05 and the number of multiple tests, n = 124, the corrected alpha, αC = α/n*rank, range is from 0.0004 to 0.05. By comparison, the Bonferroni correction would reject all with p<0.0004.

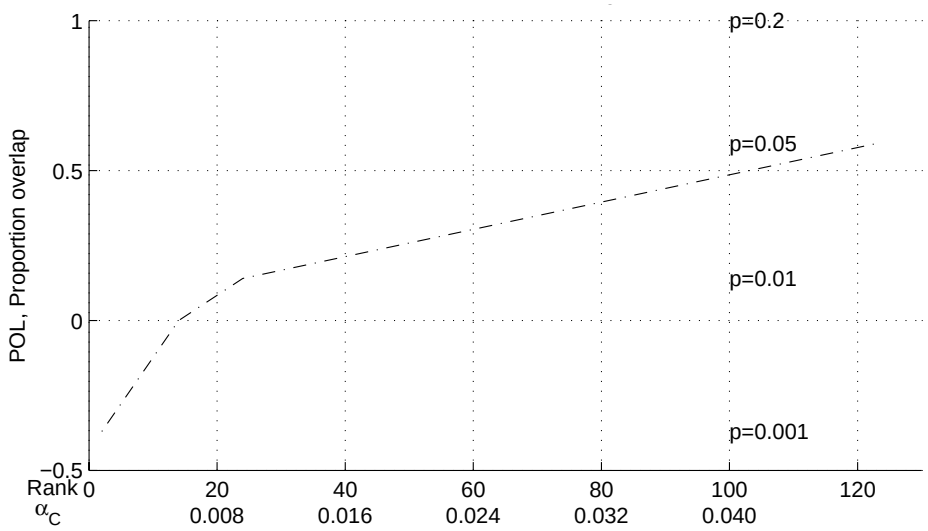


Figure 6-1 False discovery rate method for multiple tests rejects the null hypotheses where the p-value < corrected alpha. Tests are ranked in order of POL (proportion of overlap of confidence intervals); the right axis shows key p-values for POL. The corrected alpha are shown for α = 0.05 and n = 124.

6.4 Results

6.4.1 Correlations between indices

Normality test

Statistical examination of the third and fourth moments, skew and kurtosis, of the indices (example of Resting database in Figure 6-2), showed most indices were positively skewed and platykurtic.

The Shapiro-Wilk (and Shapiro-Francia) test provided evidence (Table 6-2) that forty-four of seventy indices were non-normal ($p<0.05$).

Because of the large number of indices with non-normal distributions, Spearman's rank correlation coefficient was used to determine all correlations between indices. Log transforms of indices, commonly used in HRV analysis [152] before statistical comparisons, leave the rank (and Spearman's rho) unchanged so were not used here.

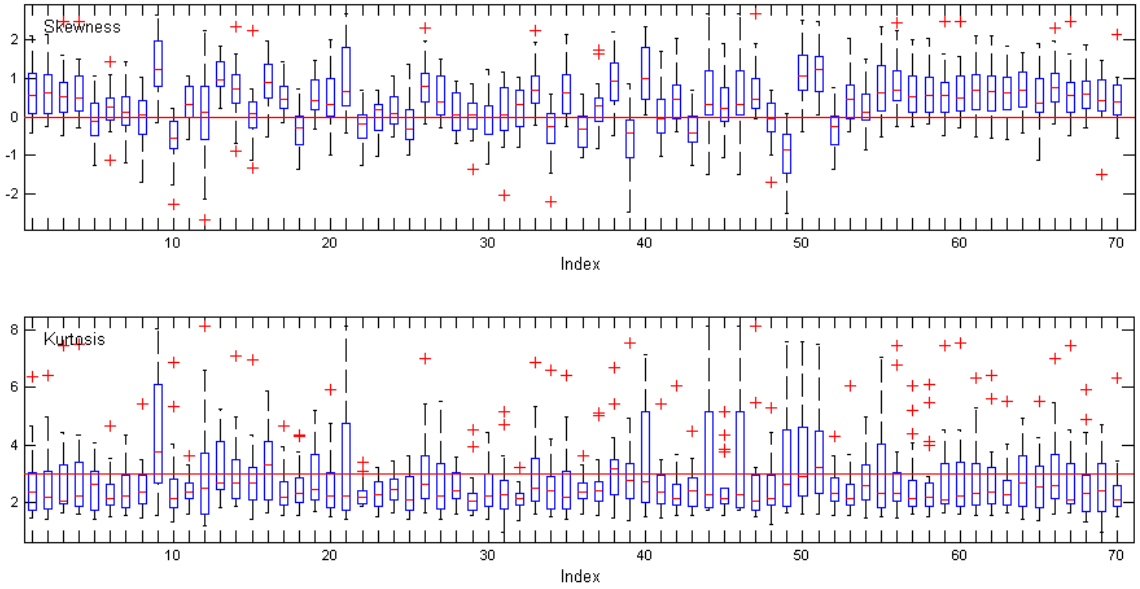


Figure 6-2 Skew and kurtosis for indices using Resting database: horizontal lines show normal skew 0 and kurtosis 3. For most of these indices skew >0 and kurtosis <3.

Table 6-2 Normality of indices with Shapiro-Wilk or Shapiro-Francia tests

Index	Reject H_0	p	SW stat	Index	Reject H_0	p	SW stat
accel	0	0.291	0.969	pQc	0	0.406	0.975
acv0x	0	0.766	0.986	pQd	0	0.819	0.986
area	1	0.030	0.967	r(1 to 25)max	1	0.001	0.713
assym(R/L)	1	0.000	0.529	r(1)-r(25)	1	0.001	0.730
CSI	0	0.131	0.951	r(RR)	1	0.024	0.898
CTMdRR	0	0.564	0.984	rmax-rmin	1	0.001	0.719
CVdRR	1	0.005	0.822	RMS	0	0.677	0.983
CVRR	1	0.026	0.901	RMSSD	1	0.005	0.831
decel	0	0.291	0.969	RMSSDmc	1	0.007	0.840
DNNEXP	1	0.000	0.264	RSA 5RR	1	0.041	0.917
grad5max	1	0.010	0.862	RSA 5RRmc	1	0.034	0.911
grad5min	1	0.023	0.896	RSA meanAD	1	0.006	0.833
gradRR	0	0.412	0.975	RSA medAD	1	0.004	0.807
kurt dRR	1	0.001	0.741	RSA PkValley	1	0.018	0.887
kurtRRz	0	0.280	0.980	RSA PVtone	1	0.001	0.689
Lomb HFnu	0	0.215	0.962	SD1	1	0.005	0.830
Lomb LF	0	0.583	0.984	SD1nu	1	0.006	0.840
Lomb LF/HF	1	0.004	0.815	SD2	1	0.034	0.911
Lomb LFnu	0	0.215	0.962	SD2nu	1	0.022	0.895
LombTotal	0	0.293	0.969	SDLD10	1	0.015	0.879
LombHF	1	0.046	0.922	SDLD4	1	0.037	0.914
magn(dRR)	1	0.006	0.833	SDLD8	1	0.017	0.884
mean.r(L1-6)	0	0.893	0.988	SDNN	1	0.034	0.912
meanRR	0	0.674	0.983	SDNN/RMSSD	0	0.079	0.938
medRR	0	0.643	0.982	SDNN/SDSD	0	0.078	0.937
NN50	1	0.001	0.735	SDNNmc	1	0.026	0.901
norm.dRR	1	0.026	0.902	SDSD	1	0.005	0.830
normRR	0	0.052	0.925	sign(dRR)	0	0.743	0.985
pNN20	1	0.001	0.944	skew.dRR	1	0.009	0.858
pNN30	1	0.000	0.916	skewRRz	0	0.952	0.988
pNN50	1	0.001	0.735	TACI(10)	0	0.400	0.982
pNN6.25	1	0.002	0.772	TACI(20)	1	0.001	0.946
PolVar20	1	0.000	0.761	TINN8	1	0.049	0.970
pQa	0	0.468	0.983	Triang8	0	0.204	0.978
pQb	1	0.001	0.946	VarIndex	1	0.008	0.852

Notes: H_0 = 1, reject null hypothesis that distribution is normal; p, significance; SW stat, test statistic.

Minimum set of indices

A minimum set of 27 indices were not correlated with the commonly used time and spectral domain indices, or were selected to represent a group of indices correlated with each other (Table 6-3). Five commonly cited standard indices have been included in this table: **SDNN**, **RMSSD**, **pNN20**, **LombHF** and **LombLF/HF**.

Correlated indices

The indices that were correlated with the standard time and spectral domain indices were removed from further analysis are displayed in Table 6-4. Also removed from further analysis with the exception of one in each group, were indices correlated with each other. Where the maximum correlation, in the middle column, is with a lesser known index, the last column gives the correlation for the more widely used standard indices. These second correlations are also >0.9.

The **pNNxx** family were all strongly correlated with each other and **RMSSD** over short windows, as were length and width of Poincaré plot, **SD1** and **SD2**.

All **RSA** indices were strongly correlated with either **SDNN** or **RMSSD**, not just with **RMSSD** the index of vagal activity. The magnitude of **RSA** (measured by peak-valley) has been shown to be a vagal measure [147] equivalent to **HF** and **RMSSD** for periods from 5 min to 1 hr [174].

Most Poincaré plot indices (length, width and area) were strongly correlated to time domain indices. The lagged plot indices, **SDLDx** were correlated to **SDNN**.

The correlations between ratios **SDNN/RMSSD**, **CSI** and **LF/HF** have been shown to be correlated from periods of 10 min down to 64 s, but not below this [290].

Table 6-3 Minimum set of indices not correlated with each other, Spearman's rho <0.9 (p<0.0001) with the addition of commonly used time and spectral domain indices (bold)

Index	Units	Highly correlated	rho
1: **SDNN**	ms	SDNNmc	0.96
2: SDNNmc			
3: **RMSSD**	ms	RMSSDmc	0.96
4: RMSSDmc			
5: **pNN20**†	%	RMSSD	0.98
6: Lomb LF	ms^2		
7: **LombHF**	ms^2	Lomb HFnu	0.89
8: Lomb HFnu			
9: **Lomb LF/HF**		Lomb HFnu	0.97
10: LombTotal	ms^2		
11: accel			
12: acv0x†			
13: assym(R/L)			
14: CVdRR			
15: gradRR			
16: kurtRRz			
17: mean.r(L1-6)			
18: meanRR	ms		
19: norm.dRR			
20: normRR			
21: PolVar20†	%	RMSSD	0.95*
22: pQa	%		
23: pQb	%		
24: pQc	%		
25: r(RR)			
26: SDNN/RMSSD			
27: skew.dRR			
28: skewRRz			
29: sign(dRR)†			
30: TACI(10)			
31: TACI(20)†			

†Average percent of zeros in data: pNN20 12%, acv0x 32%, PolVar20 43%, sign(dRR) 9%, and TACI(20) 14%.
* Correlation invalidated by outliers

Table 6-4 Indices correlated with others Spearman's rho>0.9 (p<0.001)

Index	Units	Max correlation	rho	High correlation	rho
32: area	ms^2	TINN8	0.98	SDNN	0.98
33: CSI		SDNN/RMSSD	1.00		
34: CTMdRR		VarIndex	0.99	RMSSDmc	0.99
35: CVRR		SDNNmc	1.00		
36: decel		accel	1.00		
37: DNNEXP		RSAmedAD	0.98	RMSSD	0.96
38: grad5max		RSA PVtone	0.97	RMSSDmc	0.95
39: grad5min		grad5max	0.96	RMSSDmc	0.95
40: kurt dRR		skew.dRR	0.92		
41: Lomb LFnu		Lomb HFnu	1.00		
42: magn(dRR)		RMSSD	1.00		
43: medRR	ms	meanRR	1.00		
44: NN50†		pNN50	1.00	RMSSD	0.98
45: pNN30†	%	pNN20	0.99	RMSSD	0.99
46: pNN50†	%	NN50	1.00	RMSSD	0.98
47: pNN6.25†	%	RMSSDmc	0.97		
48: pQd	%	pQa	0.94		
49: r(1)-r(25)		SDNN	1.00		
50: r(1 to 25)max		SDNN	0.98		
51: r(max)-r(min)		SDNN	0.99		
52: RMS	ms	meanRR	1.00		
53: RSA meanAD	ms	RMSSD	1.00		
54: RSA medAD	ms	RSA meanAD	0.99	RMSSD	0.99
55: RSA PkValley	ms	SDLD4	0.97	SDNN	0.95
56: RSA PVtone	ms	RMSSD	0.97		
57: RSA 5RR	ms	SDNN	1.00		
58: RSA 5RRmc	%	SDNNmc	1.00		
59: SD1	ms	RMSSD	1.00		
60: SD1nu		RMSSDmc	1.00		
61: SD2	ms	SDNN	1.00		
62: SD2nu		SDNNmc	0.99		
63: SDLD4	ms	SDLD8	0.98	SDNN	0.97
64: SDLD8	ms	SDLD10	0.99	SDNN	0.98
65: SDLD10	ms	SDLD8	0.99	SDNN	0.98
66: SDNN/SDSD		SDNN/RMSSD	1.00		
67: SDSD	ms	RMSSD	0.96		
68: TINN8	ms	SDNN	0.99		
69: Triang8		TINN8	0.99	SDNN	0.98
70: VarIndex	%	RMSSDmc	1.00		

†Average percent of zeros in data: NN50 44%, pNN30 22%, pNN50 42%, pNN20 12%, pNN6.25 40%

Visual inspection of correlations

Those indices showing a monotonic trend with Spearman's rho[8] between 0.9 and 0.95 were visually inspected to check the relationship of the data (Figure 6-3). One of these, **PolVar20**, was found to be nonlinear with multiple outliers, so the correlation was ignored and this index was included in the minimum set of indices. The other indices had valid data relationships.

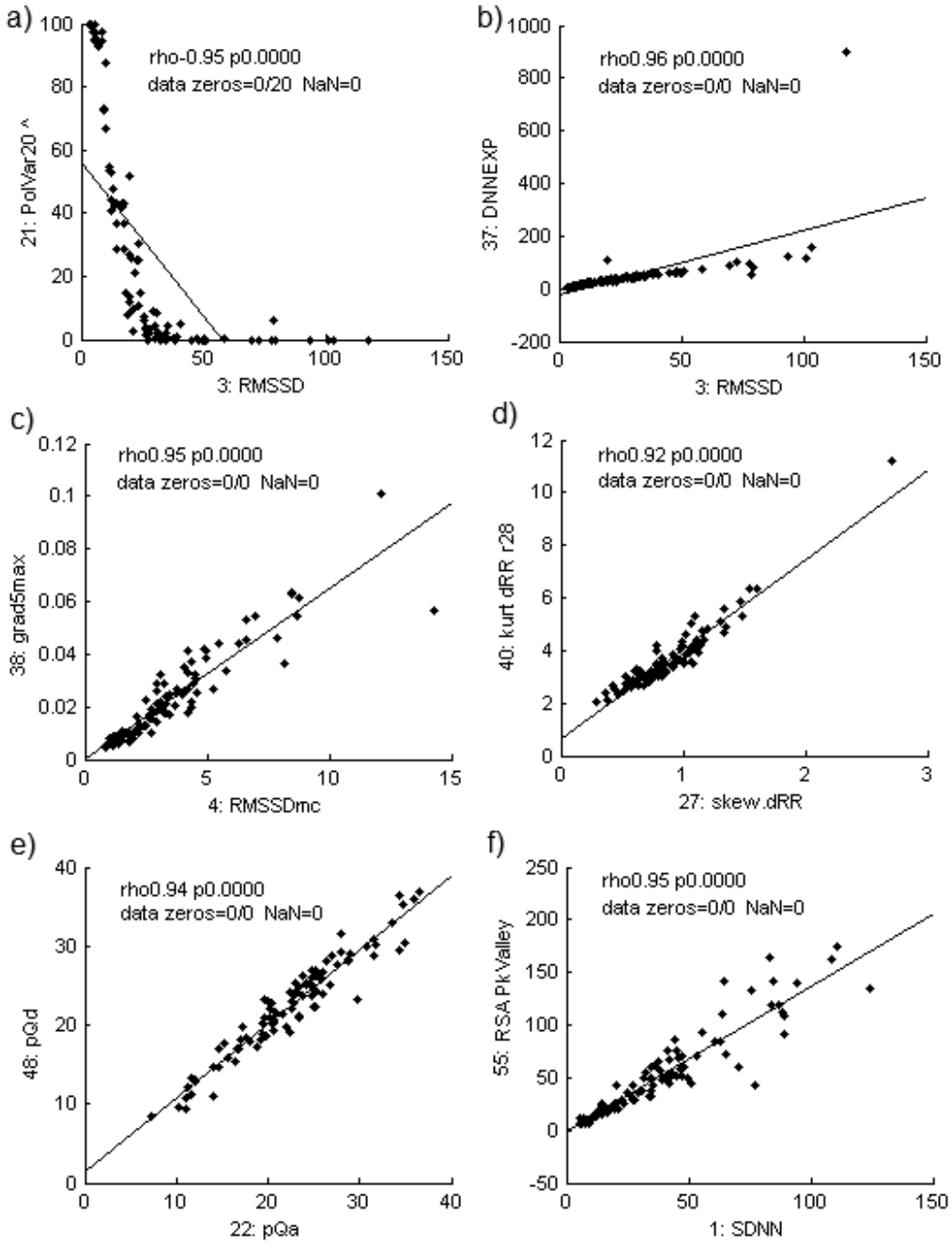


Figure 6-3 Visual inspection of correlations between indices, rho < 0.96 a) PolVar20, b) DNNEXP, c) grad5max, d) kurt dRR, e) pQd, f) RSA PkValley

[8] Spearman's rho detects a monotonic trend compared to a linear trend detected by Pearson's r.

Comparison with literature

Standard time and spectral methods all have a close mutual correlation for long-term 24 hr analysis [152, 167]. The Task Force [152] note there is correspondence within:

a) Short-term components; HF with **RMSSD**, **SDSD**, **NN50**, **pNN50**, differential index (not used here), and logarithmic index (**DNNEXP**), and

b) Overall HRV; total spectral power with **SDNN**, HRV triangular index (**TRIANG**) and **TINN**.

Other studies which provided correlation values are shown in Table 6-5.

This study agreed with the mutual correlations within the time domain indices but did not find correspondence between spectral and time domain indices for the 30-beat period.

Table 6-5 Correlations between indices: comparison with literature for longer periods (5 min to 24 hr) for normal subjects [318, 399] and patients after myocardial infarct [400, 401]

Index 1	Index 2	r literature	rho 30 beats
RMSSD	pNN50	0.99 [400] 0.94 [318] 0.96 [399]	0.98
LF	Total Power	0.91-0.93 [318, 399]	0.47
SDNN	Total Power	0.85-0.87 [399, 400] 0.96 [318, 402]	0.41
RMSSD	HF power	0.87 [400] 0.97 [318] 0.88-0.98 [205]	0.35
HF	Total Power	0.98 [318] 0.88 [399]	0.32
SDNN	LF power	0.51-0.83 [205] 0.85 [399]	0.25

Abbreviations: r, Pearsons correlation coefficient; rho, Spearman's rank correlation coefficient.

6.4.2 Correlation with mean heart rate

The correlation (Spearman's rho) between each index and the mean heart rate was used to assess indices that may be influenced by the most common inter-individual difference (i.e. heart rate) and determine if indices need further normalisation or correction for mean heart rate. The mean RR-interval, inverse of heart rate, was used for this assessment.

The indices with the highest correlation with mean RR-interval, shown in Figure 6-4, have a moderate correlation with heart rate that needs to be taken into consideration when analysing results. It is also possible that these are not directly correlated with heart rate, but are more likely to occur in conditions of slow (**pNN20**) or fast (**PolVar20**) heart rate.

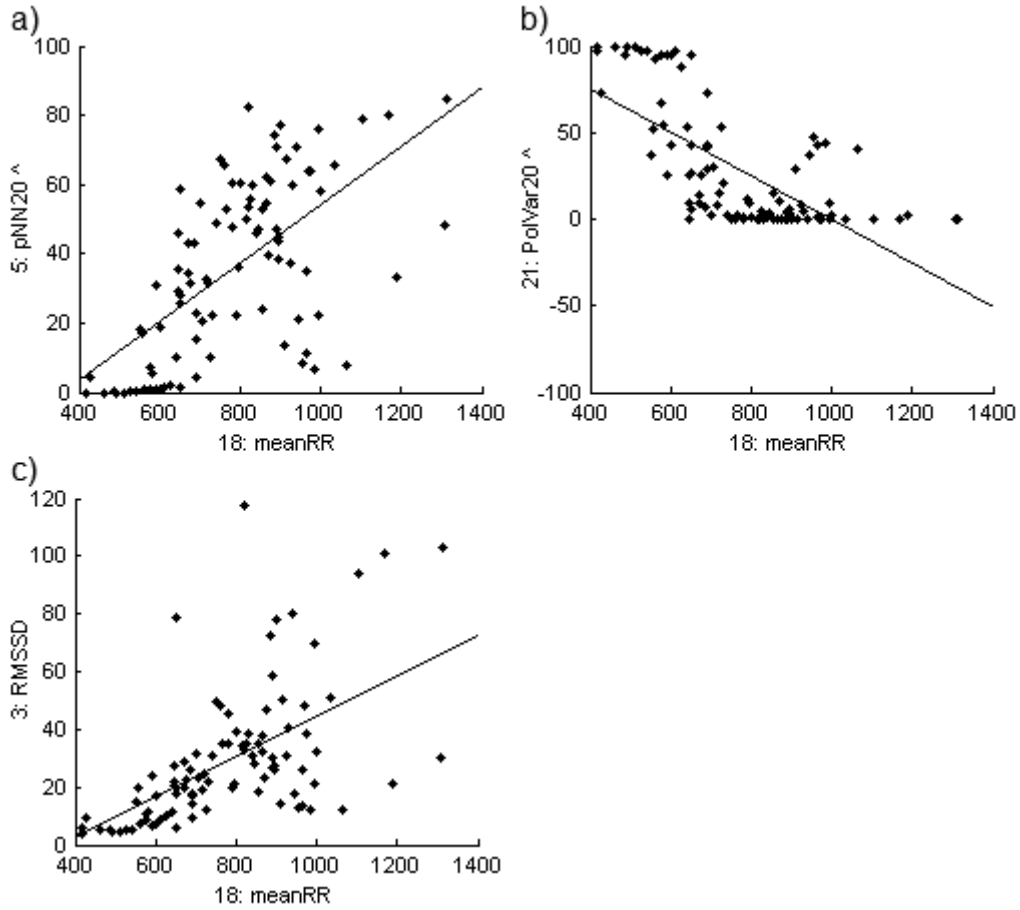


Figure 6-4 Indices and mean RR-interval with highest correlations (rho >0.60, p<0.0001) a) pNN20, rho -0.65, b) PolVar20, rho 0.68, c) RMSSD, rho -0.64 (average for each subject in 5 databases)

6.4.3 Stationarity of data

StatAv near zero indicates stationarity, whereas larger values (near 1) indicate greater nonstationarity [403].

The mean corrected stationarity index, StatAvc [382], was not used as StatAv for this data was not related to the mean (Table 6-6). Only the Active data shows a moderate inverse correlation over 300 beats (rho = -0.59 p = 0.006).

Table 6-6 Stationarity test a) Correlation of StatAv with mean RR-interval (Spearman's rho) and b) StatAv of 300 and 30-beat segments for each database

	Resting	Active	Exercise	Sleep	Meditation
a) Correlation of StatAv with mean RR-interval, rho					
300 beat	-0.08	-0.52*	-0.13	-0.05	-0.24
30 beat	-0.01	-0.2	0.07	0.18	0.00
b) StatAv 300 beats					
Mean (range)	0.53 (0.28-0.81)	0.70 (0.35-0.98)	0.97 (0.55-1.0)	0.43 (0.23-0.65)	0.34 (0.2-0.58)
<0.5	50%	15%	0%	56%	92%
StatAv 30beats (x10)					
Mean (range)	0.71 (0.47-0.93)	0.78 (0.50-0.97)	0.8 (0.53-1.0)	0.8 (0.59-0.95)	0.84 (0.70-0.96)
<0.5	17%	10%	8%	4%	7%

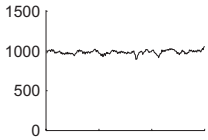
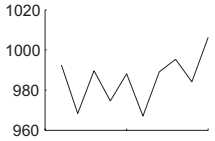
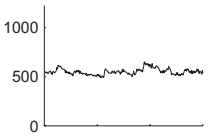
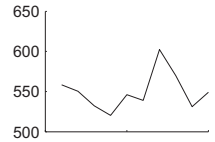
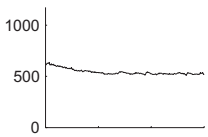
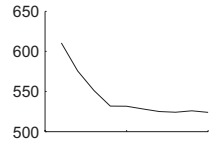
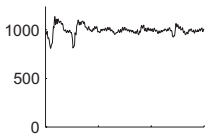
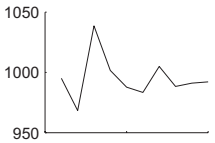
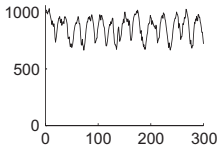
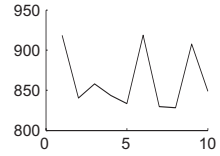
* p<0.1 (p = 0.02)

The longer 300 beat records are more stationary than short 30-beat records (Table 6-7). With 300 beat segments, the most stationary data measured by StatAv was in the meditation database where 11 of 12 subjects had stationary signals. Exercise data was entirely non-stationary for all 20 subjects.

For 30-beat segments, only a small subset in any one database was stationary. The most stationary data were in the Resting database with 17% of subsets exhibiting stationarity.

For this study, nonstationarities were not removed.

Table 6-7 Database stationarity examples: RR-interval tachogram and StatAv for one subject in each database

Database (subject)	RR-interval 300 beats	Mean RR-interval 30 beats (x10)	StatAv 300 beats	StatAv 30 beats (range)
Resting (f1o01)			0.5	0.8 (0.7-1.0)
Active (m16265)			0.8	0.8 (0.5-1.0)
Exercise (ex311)			1.0	0.8 (0.6-1.0)
Sleep (slp14)			0.4	0.8 (0.6-1.0)
Meditation (C1med)			0.4	1.0 (0.9-1.0)

Note: For stationary signals, StatAv = 0. StatAv: for 300 beats divided into 10 x 30 beat segments; for 30 beats (x10) each is divided into 6 x 5 beat segments.

6.4.4 Establishment of CI

Confidence intervals were established for the selected indices over five different physiological states using bootstrapped 95% confidence intervals for the median (Table 6-8). Plots of the 95% confidence levels for a selection of the indices differentiating the databases are shown in Figure 6-7.

The 300 beat periods vary from 2.3 min (exercise) to 6.1 min (sleep) with mean RR-interval 465.5 ms and 1222 ms respectively.

6.4.5 Correction for multiple tests

Correction for multiple tests (Figure 6-5) allowed 80 occurrences where a difference was detected between databases and Resting.

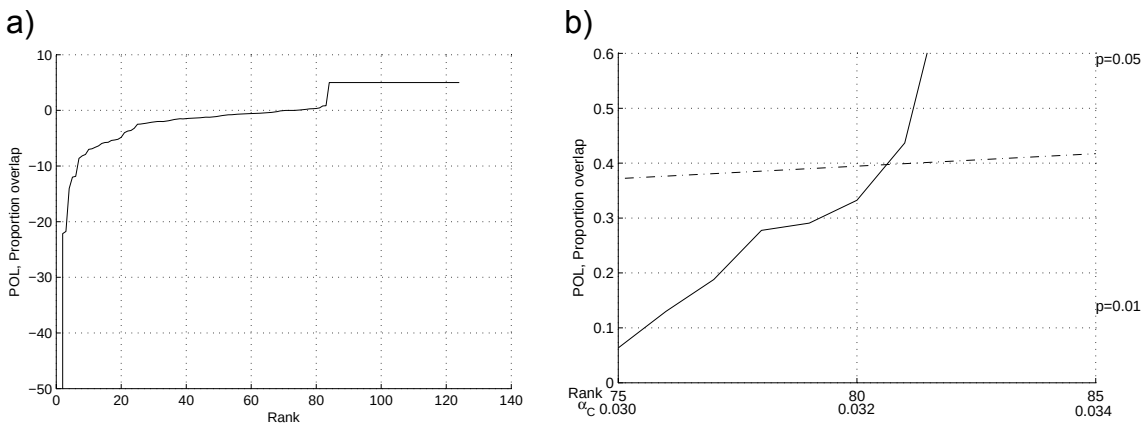


*Figure 6-5 Ranked proportion overlap (POL) of confidence intervals between physiological states a) all 124 tests (31 indices 4 database comparisons for each), b) decision area, x-axis gives rank and associated FDR (rank * alpha/number of tests) with alpha = 0.05. Effective rejection of null hypothesis, H0, that there is no difference for POL <=0 (i.e. no overlap between confidence intervals).*

Table 6-8 Bootstrap 95% confidence intervals for median of indices for 10x30 beat segments.

Index	A. Resting	B. Active	C. Exercise	D. Sleep	E. Meditation	p
1: SDNN	30.9–38.8	26.5–34.5	7.94–9.90	34.9–44.9	55.5–75.6	c,e
2: SDNNmc	3.25–4.10	3.82–4.63	1.46–1.71	4.41–5.87	7.74–9.26	c,d,e
3: RMSSD	27.7–33.5	20.8–26.2	5.90–7.10	27.0–31.8	30.7–38.0	b,c
4: RMSSDmc	2.65–3.67	2.99–3.26	1.12–1.33	3.47–3.99	3.91–4.60	c,e
5: pNN20 ^	41.4–51.7	29.3–41.4	0.00–0.00	37.9–48.3	48.3–58.6	b,c
6: Lomb LF	19.4–24.1	24.7–28.6	22.4–25.6	20.5–23.9	39.5–43.3	b,e
7: LombHF	15.5–19.9	9.21–12.15	7.72–10.02	11.8–15.7	5.28–6.81	b,c,d',e
8: Lomb HFnu	39.1–47.9	26.0–31.3	24.9–33.6	31.9–40.6	11.1–15.0	b,c,d',e
9: Lomb LF/HF	1.09–1.58	2.19–2.85	1.98–3.02	1.46–2.13	5.70–8.04	b,c,e
10: LombTotal	42.7–46.2	37.9–41.6	34.0–38.3	37.8–41.7	45.7–48.9	b,c,d
11: accel	0.49–0.53	0.48–0.52	0.44–0.47	0.46–0.51	0.49–0.58	c
12: acv0x	2.00–3.00	2.50–4.00	0.00–1.00	2.00–2.00	4.00–4.00	c,e
13: assym(R/L)	0.70–0.97	1.04–1.40	0.96–1.20	1.19–1.73	1.30–2.04	b,d,e
14: CVdRR	124–126	127–130	126–129	126–129	126–130	b,d,e
15: gradRR	-0.23–0.48	-0.00–0.77	-0.42–-0.25	-0.60–0.33	-0.92–0.77	c
16: kurtRRz	2.44–2.60	2.44–2.66	2.32–2.50	2.31–2.56	1.95–2.28	e
17: mean.r(L1-6)	0.07–0.14	0.16–0.25	0.24–0.34	0.22–0.31	0.16–0.25	b,c,d,e
18: meanRR	965–988	694–738	540–580	816–851	731–820	b,c,d,e
19: norm.dRR	0.12–0.13	0.14–0.15	0.13–0.15	0.12–0.14	0.14–0.16	b,c,e
20: normRR	0.12–0.14	0.14–0.16	0.14–0.16	0.13–0.14	0.15–0.16	b,c,e
21: PolVar20 ^	0.00–0.00	0.00–10.42	100–100	0.00–0.00	0.00–0.00	b,c
22: pQa	25.0–26.9	21.1–23.1	23.1–26.9	20.0–22.2	11.5–15.4	b,d,e
23: pQb	20.0–22.2	19.0–24.0	11.3–13.2	25.9–29.2	23.1–27.1	c,d,e
24: pQc	18.2–20.4	15.4–18.5	16.0–19.0	23.1–26.1	30.0–37.5	b',d,e
25: r(RR)	0.50–0.60	0.64–0.72	0.65–0.73	0.68–0.77	0.83–0.89	b,c,d,e
26: SDNN/RMSSD	1.02–1.16	1.20–1.36	1.21–1.40	1.29–1.49	1.75–2.10	b,c,d,e
27: skew.dRR	0.69–0.87	0.74–0.87	0.51–0.70	0.74–1.00	0.68–0.95	c'
28: skewRRz	-0.19–-0.01	0.01–0.22	-0.02–0.15	0.12–0.32	0.09–0.29	b,d,e
29: sign(dRR) ^	-1.00–1.00	0.00–2.00	-2.00–-1.00	0.00–1.00	-2.00–0.00	c
30: TACI(10)	0.44–0.50	0.46–0.54	0.50–0.60	0.33–0.40	0.43–0.57	c,d
31: TACI(20)	0.44–0.50	0.45–0.56	0.00–0.00	0.38–0.45	0.41–0.50	c,d’

^ corrected percentile method of bootstrap, otherwise bias corrected and accelerated percentile method
p<0.006 (or ‘p<0.04) for b:AvB, c:AvC, d:AvD, and e:AvE

6.4.6 Comparison of baselines with literature

Resting compared to normative

A comparison of the Resting data and normative data are shown in Table 6-9. Normative data [404] are only available for a few of the indices, from short-term (3 min to 2hr, mainly supine) studies with free breathing.

The Resting data used in this study (with 30-beat windows) have similar heart rates and **HFnu** compared to normative data, but lower **SDNN**, **RMSSD** and **LF/HF** (Table 6-9). A lower value for **SDNN** is expected as the variance is known to decrease with shorter recording times [274, 405]. The **HF** value cannot be compared as the magnitude also changes with the length of the window.

Table 6-9 Comparison of Resting data with normative values for free-breathing short-term (5 min to 2 hr) studies

Index	A. Resting 30-beat 95% CI	Normative 5 min-2 hr Mean (SD)
Mean RR	964–988	926 (90)
SDNN	31–39	50 (16)
RMSSD	27–34	42 (15)
HF	16–20	657 (777)
HFnu	39–48	40 (10)
LF/HF	1.1–1.6	2.8 (2.6)

Active

The Active data (compared to Resting) showed: a decrease in **meanRR**; a decrease in vagal activity **RMSSD** (but not **RMSSDmc**), **pNN20, LombHF** and **LombHFnu**; a small increase in sympathetic activity **LombLF**, and sympathovagal balance, **LombLF/HF.**

These are all in agreement with published data. Changing posture from supine to sitting or standing is known to cause a decrease in RR-interval, a modest increase in LF, a large decrease in HF, and an increase in LF/HF [406-408] also increases in LFnu and decreases HFnu [409], and decreased RMSSD [410].

Some indices only decrease on standing (and not sitting) such as SDNN and total power [409].

Exercise

In exercise vagal tone is reduced and sympathetic activity is increased. This is shown by a decrease in the time domain indices, **SDNN** and **RMSSD** [180, 411-413]. The heart rate increased as expected with a decrease in **meanRR** to 569-632 ms (30-beat window of 17-19 s) and there were corresponding decreases in **pNN20** and increases in **PolVar20**.

Vagal withdrawal at the onset of exercise is generally shown by decreased respiratory sinus arrhythmia (RSA) indices [414-416] that in this study are correlated with **SDNN** and **RMSSD**.

Contrary to what may be expected, spectral analysis does not reflect the activation of the sympathetic system and decrease in vagal activity with increasing loads in exercise [411, 417]. Usually spectral analysis shows a decrease of total, and LF power [154, 411, 413, 416, 418] though LF power may remain steady for sub-maximal exercise, especially with older sedentary females, and HF power tends to reduce in low intensity tasks [417]. The results here showed a decrease in **LombHF**, **LombHFnu** and **LombTotal** and no change for **LombLF**. The sympathovagal balance indices, **SDNN/RMSSD** and **LombLF/HF**, were increased [290, 418].

Sleep

Compared to Resting (in a supine position), Sleep showed a decrease in **LombHF, LombHFnu**, and **LombTotal**, an increase in **SDNNmc** and **LombLF/HF**, and there was no change in **RMSSD** and **pNN20**.

However, compared to Active (rather than Resting), **meanRR**, **SDNN, RMSSD** and **LombHFnu** increased, with decreased **Lomb LF** and **LombLF/HF**, all consistent with a night-time increase in parasympathetic activity and a decrease in sympathetic activity [272, 419-421].

For Stage 2 sleep, Otzenberger *et al.* [205] found lower **r(RR)** compared to intrasleep waking periods, and correlation of **r(RR)** with lower **LombLF/HF** but they did not give information on the actual values.

In the Sleep database used here, the subjects were being investigated for sleep apnoeas and arousals. Comparison with Narkiewicz *et al.* [422] may be more appropriate. They found patients with moderate to severe obstructive sleep apnoea, compared to controls, had a shorter meanRR of 793 ±27 ms lower than that seen here, with reduced HFnu, and increased LF/HF that were seen here. They also saw increased LFnu.

Our study showed increased **pQb** and **pQc** as found by Raetz *et al.* [202] with sleeping cats in quiet sleep.

Different results may be expected because sleep is difficult to analyse as large changes can occur over periods as small as 5 min [423] depending on sleep stage, with large changes immediately after sleep onset [424] and between slow wave and rapid eye movement sleep stages [425].

Meditation

Meditation increased total HRV as shown by **SDNN** and short-term variability **RMSSD**. Heart rate decreased (for **meanRR** 733-825 ms, 30-beat window is 22-25 s) but this can be higher or lower than resting depending on the type of meditation [426]. Meditation caused the expected increase in **LombLF/HF** [426], and showed the paradoxical decrease in **LombHF** spectral power that has been noted previously [427, 428]. This is due to the slow breathing rate moving below 0.15 Hz, out of the traditionally defined HF range and into the LF range (Figure 6-6).

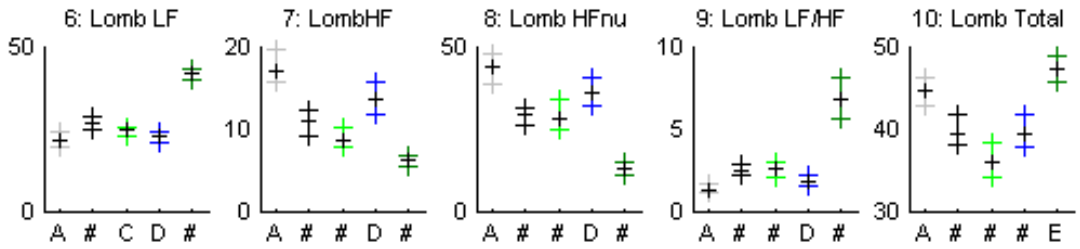


Figure 6-6 Lomb-Scargle spectral indices show paradoxical change in meditation (i.e. increase in LF and decrease in HF), that occurs due to slowed breathing moving the power out of the traditionally defined HF range. Database label (B-E) replaced with # p<0.006 significant difference from A.Resting to B.Active, C.Exercise, D.Sleep or E.Meditation.

Meditation had a large increase in sympathovagal balance **SDNN/RMSSD**, with corresponding changes in the Raetz quadrant indices: more RR-intervals as part of a run of increases or decreases, **pQb** and, **pQc** respectively, and fewer changed from decrease to increase, **pQa**.

6.4.7 Usefulness of indices

All indices differentiated at least one physiological state from Resting:

- Five indices were only able to differentiate Exercise from Resting: **accel**, **gradRR**, **skew dRR**, **sign(dRR)** and **TACI(20)**.
- One index, **kurtRRz**, could only differentiate Meditation from Resting.
- Eight indices could differentiate all four states from Resting (but not necessarily from each other): **LombHF**, **assym(R/L)**, **CVdRR**, **mean.r(L1-6**), **meanRR**, **r(RR)**, **SDNN/RMSSD**, and **skewRR**.

Correction for multiple tests using the False Discovery Rate removed none of the 80 tests that had been identified as significant ($p<0.05$) but kept the likelihood of Type II errors to a minimum.

Visual examination of confidence interval plots (Figure 6-6 and Figure 6-7) shows similarities in the response of some indices: **SDNN/RMSSD** is similar to **LombLF/HF**, **LombTotal** and inverse **mean r(L1-6)**, **pNN20** and inverse **PolVar20**.

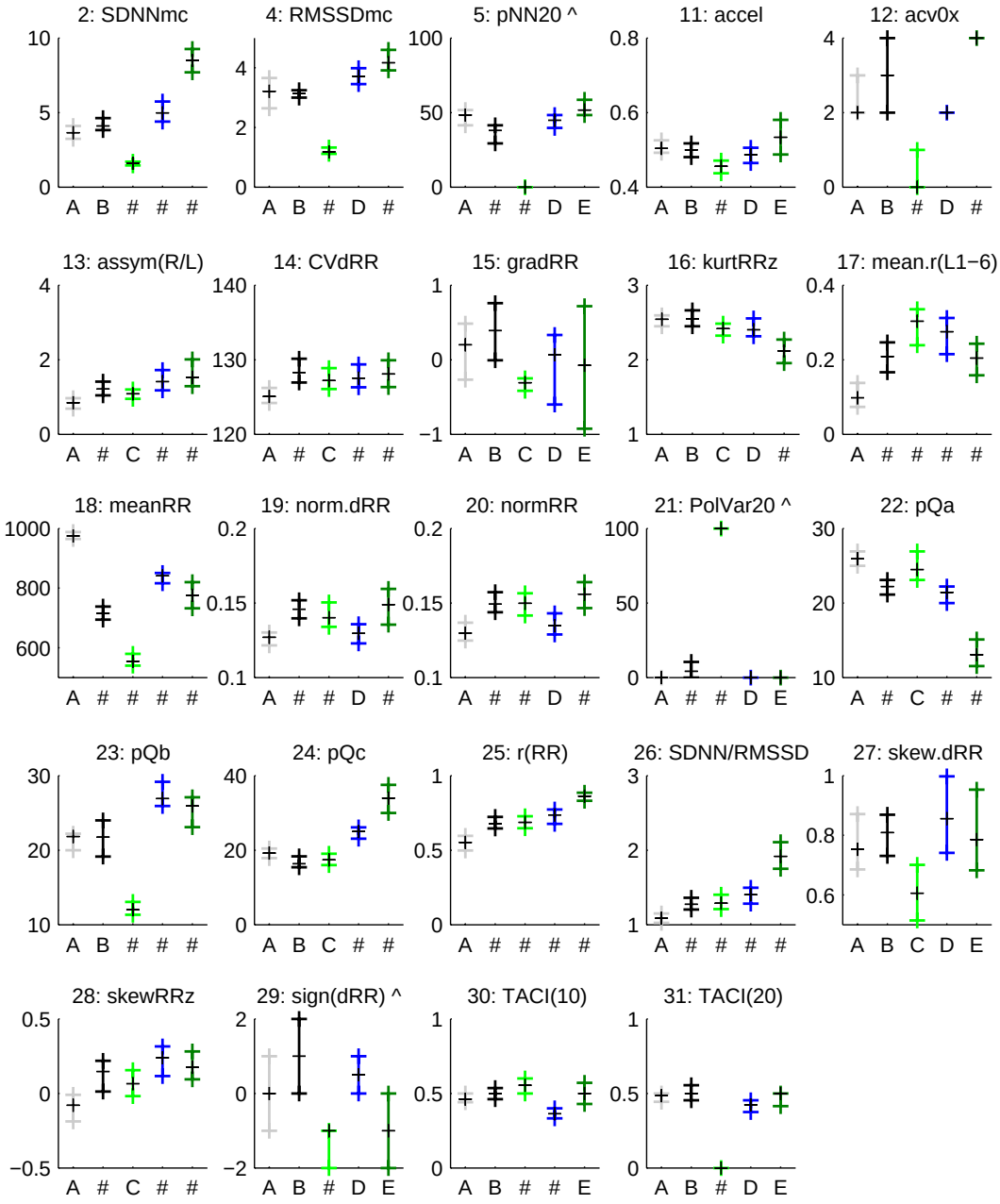


Figure 6-7 Physiological database baseline 95% confidence intervals of the median with bootstrap resampling (10,000) for 10x30 beat windows. Database label (B-E) replaced with # p<0.006 significant difference from A.Resting to B.Active, C.Exercise, D.Sleep or E.Meditation.

6.5 Discussion

6.5.1 Correlations of indices

This study used a selection of indices beyond the standard time and spectral domain indices that may be useful in short-term analysis of autonomic nervous system activity. The indices included less well known measures of heart rate fluctuations and characteristics.

HRV data is usually non-normal requiring log transforms of the data or non-parametric methods of statistical analysis [185, 318, 339, 429]. The non-normality was confirmed here in short 30-beat windows requiring non-parametric determination of correlation coefficients.

Indices that were correlated over a variety of physiological states were removed from the minimum set of indices, except for those commonly used in HRV studies. These time domain and spectral indices are known to be correlated over longer 256 beat periods [156, 197]. The use of a range of physiological states reduced the likelihood of removing an index that was specific to one state [270]. These correlations could be better tested over the full range of physiological states by including data with pharmacological blockade of the autonomic nervous system [430, 431]; however these data were not available for this study.

All **RSA** indices and most Poincaré plot indices (length, width and area) were strongly correlated to time domain indices. Some indices excluded from this study (e.g. **pNNxx)**, may still be relevant in studies of known high vagal activity (e.g. during sleep).

Some **RSA** indices were more highly correlated with **SDNN** over short periods rather than with **RMSSD** the index of vagal activity as would be expected.

6.5.2 Stationarity

Stationarity measured by StatAv was rare in 30-beat windows; exercise was the least stationary with obvious linear trends throughout the data for each subject.

Nonstationarities are usually undesirable and are removed from data because they reduce effectiveness of spectral analysis caused by data smearing [432].

Nonstationarities were not removed for two reasons a) linear trends have only a small effect over 30-beat periods (Figure 5-16), and b) the nonstationarities may contain dynamic data.

The removal of stationarities from HRV data, which is known to be nonstationary, is questioned by some authors [285, 375, 433-435].

Yum *et al.* [435] pointed out that if the non-stationarity is related to non-neural heart rate regulation, non-stationarity analysis will provide information about both neural and non-neural regulation of heart rate and the short-term non-stationarity provides a way of characterising HRV.

Pinna *et al.* [375] also comment that a trend in the data may be part of the random fluctuation of the underlying process, with frequency components longer than the data length. In this case, trend removal procedures act as high-pass filters.

6.5.3 Establishment of CI

The confidence intervals established for each of the indices were consistent with literature for longer samples; the increases and decreases are generally in agreement with published results. The magnitude for shorter baseline periods can only be directly compared for indices that are normalised.

6.5.4 Usefulness of short-term indices

Different physiological states

This study shows that both standard indices and other less-used HRV indices are capable of differentiating different physiological states over periods as short as 30-beat intervals. All indices differentiated at least one physiological state from Resting.

The states with increased sympathetic activity (Active and Exercise) showed expected increases in sympathetic activity (**LombLF** and **LombLF/HF**), decreases in total variability (**LombTotal**), and decreases in vagal indices

(**pNN20**, **LombHF**, and **LombHFnu**). Decreased **RMSSDmc** and **SDNNmc** indicative of vagal withdrawal only occurred with Exercise and not with the lesser exertion in Active.

Examination of the less commonly used indices with different physiological conditions gives new information in situations where indices have not previously been investigated: the only index that identified the higher sympathetic activity of exercise with a large positive magnitude is **PolVar20**; Meditation and Exercise have a similar (negative) effect in **sign(dRR)** but opposite effects in **pQa**; Exercise and Sleep have similar **r(RR)** but Meditation is different; Exercise and Meditation have similar **norm.dRR** but Sleep is different; Sleep and Meditation have similar **skewRRz** but Exercise is different.

Further analysis of indices with principal components analysis or cluster analysis, may find that some with a similar response can be removed from the minimum set (e.g. **SDNN/RMSSD** and **LombLF/HF**).

Short-time periods for parasympathetic activity

The usefulness of vagal tone indices has been confirmed by others with 10 s periods, shorter than the minimum 30-beat periods used here of 13.8 s (for Exercise).

Hamilton *et al.* [210] have shown that vagal tone can effectively be measured in 10 s by two vagal measures: **RMSSD** and AAD (average absolute difference, equivalent to **RSA meanAD** in this study), and less so by **SDNN**. **RSA meanAD** was found to be strongly correlated with **RMSSD** over 30-beat windows so was not used in the minimum set of indices.

Thong *et al.* [211] also showed 10 s **RMSSD** to be equivalent to 300 s **RMSSD** for subjects assumed to be resting (identified[9] as SDNN < 30ms) after detrending with a 21 point moving average. Observations in Chapter 5.4.5

[9] This SDNN criteria for resting may have a typo. According to our 30 beat data in Table 6-8, the test for the supine condition should have been SDNN > 30ms as this index decreases with activity and exercise 180. Tulppo MP, Makikallio TH, Takala TE, Seppanen T, Huikuri HV. Quantitative beat-to-beat analysis of heart rate dynamics during exercise. Am J Physiol. 1996 Jul;271(1 Pt 2):H244-52, 411. Casadei B, Cochrane S, Johnston J, Conway J, Sleight P. Pitfalls in the interpretation of spectral analysis of the heart rate variability during exercise in humans. Acta Physiol Scand. 1995 Feb;153(2):125-31..

showed that trend removal was not required for short term vagal analysis with spectral index **LombHF**.

Cardiac mortality has been predicted with 10 s **RMSSD** [316, 436] and 10 s **SDNN** [437], Other studies have used **RMSSD** for detecting vagal impairment in Chagas disease in 10 s [438], exercise and recovery with 25 s sliding window [439], mental stress in 15-30 s [440], and studying the effect of tongue position in 60 s [441].

Detection of vagal indices over the short-term is now accepted with the development of a variety of devices monitoring real-time cardiac vagal tone: Neuroscope (Pontoppidan, Copenhagen, Denmark) measuring CIPA, a cardiac index of parasympathetic activity [442, 443], Vagal Tone Monitor (Delta Biometrix, Bethesda MD) [171], and a hand-held photoplethysmographic device [444, 445]

Detection of spectral power indices over short term

A device has been developed (MemCalc method, Tarawa, Suwa Trust, Japan) for 30 s estimation of the power spectrum using the maximum entropy method [446]. This device has been used in many studies in Japan: studying haemodynamic fluctuation during induction of anaesthesia [447], sympathovagal effects of spinal anaesthesia [257] and the different effects of intravenous anaesthetics [448]. Sesay *et al.* [449] showed a correlation between real-time LF and plasma norepinephrine during adrenal surgery.

Detection of other indices over short term

The requirement for longer windows to detect sympathetic activity has led to development of multi-time analysis. Salahuddin *et al.* determined the minimum period that indices remained equivalent to longer periods and found it depended on both the index and the physiological state or disease being investigated; the minimum periods for mental stress [450] and atrial fibrillation [451] for a range of HRV indices are shown in Table 6-10.

Balocchi *et al.* found **SDNN/RMSSD** (similar to LF/HF) could be used to differentiate head-up tilt from rest for periods down to 64 s [290].

This study used indices over 30 beats, the period varying from 13.8 s (exercise) to 36.6 s (sleep) depending on heart rate.

Table 6-10 Summary of minimum period (s) for short-term indices

Index	Mental stress [450]	Atrial fibrillation [451]
Mean HR, meanRR	10	10
SDNN		40
RMSSD	30	40
pNN50	30	10
LF		250
HF	40	50
LF/HF, LFnu, HFnu	50	70
CV		40
TINN		80
HRV Index		60

6.5.5 Spectral analysis

It is generally recommended[10] for HRV that spectral analysis be performed on a recording at least 10 times the wavelength of the lower frequency limit, that is at least 2 min (256 to 512 beats) for the LF component [152], to ensure sufficient frequency resolution while maintaining stationarity.

Use of the Lomb-Scargle algorithms avoided the need for interpolation of data points and allowed the 30-beat periods to be evaluated without pre-processing. Lomb-Scargle analysis was able to differentiate sleep and meditation, and clearly identified the paradoxical shift in meditation from HF to LF even with 30-beat samples. Furthermore, nonstationary trends (VLF noise) were shown to have little effect on 30-beat samples.

This study found that for spectral indices over shorter periods, although the result was different from longer periods, the relative values remained the same.

[10] Note: The Task Force recommendations are confusing as the lower limit of LF at 0.04 Hz requires 250 s (i.e. 10/0.04) which is 4.17 min, twice as long as the 2 min recommendation they make. A 2 min record length would only capture a lower limit of 0.083 Hz.

6.5.6 Statistics

Correction for multiple comparisons has been made using the FDR method recommended for exploratory research. As an exploration of the use of short-term indices in different physiological states, and with no clinical implications, this study highlights indices that may be interesting to study further, and determines the size of index changes for different physiological states that can be used to determine effect sizes in subsequent studies.

6.5.7 Limitations

Indices were only tested with a small range of physiological states available in the PhysioNet databases. While the PhysioNet databases are convenient to use, there are disadvantages with using real, unsupervised, data.

Active subjects were known to have no significant arrhythmias from long term recordings (24 hr), but the start time was not consistent (8 am to 11:48 am) and there was no information regarding activity status (supine, sitting, or walking). Known periods of activity are not identified, interventions (injections, examinations) and normal daily routines (eating, ambulation, and sleep) were likely occurring intermittently through this period. For all subjects, speech was possible and may have had an effect [452].

The subjects in the Exercise database were being investigated for ST changes thus subjects may be expected to have some heart disease. No annotation was available on age or gender of participants, exercise protocol or level of exercise achieved. Meditation subjects did not have their sex and age identified.

Sample sizes were limited for Sleep (16) and Meditation (N = 12). While other databases were available for sleep, the sleep stage was not annotated. The Sleep database used here was originally for investigation of sleep apnoeas and arousals, and may not have characteristics of normal subjects.

These database limitations could be overcome with further controlled experiments to validate these short-term indices using the gold standard tests (i.e. adrenergic and vagal blockade) in the same individuals.

6.6 Summary

This study has identified a minimum set of indices for future use in dynamic sub-minute studies of HRV. Correlated indices were removed, confidence intervals were established with real data over a 30-beat window in resting and other physiological states, and results compared to longer periods in the literature.

All RSA indexes and most Poincaré plot indices (length, width and area) were related to time domain measures (SDNN or RMSSD).

The ability of indices to differentiate physiological states was tested with real data from PhysioNet databases of Resting, Active, Exercise, Sleep, and Meditation. Baseline 95% confidence intervals of the median were established with bootstrap resampling (10,000 times). Statistical significance was assessed using the overlap of 95% confidence intervals. Correlation of subject average was used to remove redundant indices and define a minimal set of indices for characterising the physiological states.

Lomb-Scargle spectral power analysis over 30-beat windows correctly identified: parasympathetic shifts in sleep; decreased spectral indices in exercise; and paradoxical shifts in meditation. Indices of 30-beat windows were able to distinguish characteristics of different physiological states that are linked to changes in sympathetic and parasympathetic nervous systems and could be useful in dynamic HRV analysis where long periods of stationary data are not available.

These indices may be useful to provide insight into dynamic changes in the autonomic nervous system, where stationarity is not attained, and effects last less than a minute such as can be expected with the administration of fentanyl.

Chapter 7. Method for studying HRV of fentanyl-induced loss of airway tone

7.1 Introduction

A pilot study was designed to use preoperative administration of fentanyl to examine the association between central nervous system pathways affecting opioid-induced loss of airway tone and cardiac activity measured by HRV.

Opioids can cause respiratory depression by decreasing rate and depth of breathing and by decreasing upper airway tone leading to airway collapse. The upper airway tone is normally controlled within each breath to stiffen the airway on inspiration and prevent collapse occurring. The heart rate is also affected by respiration with acceleration of the heart rate with inspiration and this can be measured by a variety of heart rate variability indices.

7.2 Aims

This pilot observational study investigated the progression of cardiovascular autonomic nervous system changes from opioid administration to respiratory depression using very short-term HRV measured over 30-beat intervals.

The hypothesis is that a measure of short-term, non-stationary HRV can detect a change in vagal or sympathetic activity that may be a reflection of a shift in the stability of airway tone.

It is unknown a) if very short-term HRV can detect opioid-induced autonomic nervous system changes, b) what the opioid effects on very short-term HRV are, and c) if changes to cardiac autonomic activity occur with, or precedes, critical respiratory depression.

7.3 Design of study

This study was designed to replicate the post-operative opioid effect of reduced airway tone in a subject while in a safe environment with continuous physiological monitoring and anaesthetist presence.

7.3.1 Use of fentanyl

Fentanyl acts on the same opioid receptor (mu) in the body as morphine [71, 72], so it is likely they affect the same central nervous system pathways that lead to respiratory depression. In the operating theatre setting, fentanyl is the opiate of choice. It is commonly given in moderate to large intravenous doses preoperatively [7].

Fentanyl is a semi-synthetic opioid with strong affinity for the mu opioid receptor site that produces analgesia as well as opioid adverse effects, such as nausea, sedation, and respiratory depression [453]. Other effects of fentanyl are to reduce the dose of intravenous anaesthetic agent, dull laryngeal reflexes, minimise the adverse cardiovascular consequences of instrumentation of the airway (including the endotracheal intubation), reduce respiratory drive and provide analgesia during surgery.

For many opioids, there is a significant time lag between peak concentration in the plasma and peak drug effect. By the intravenous route, fentanyl has a rapid onset of analgesia (1-5 min) and a short duration of action (less than 1 hr). Peak effect occurs within 3-5 min [453]. For intravenous fentanyl (100 µg) peak brain concentrations occur in 6 min based on EEG effects [6, 79].

In humans, the occurrence of apnoea shortly after the 90 s infusion of high-dose fentanyl (>= 200 µg) is related to the rapid increase in blood fentanyl concentration, its rapid passage across the blood-brain barrier (the fentanyl blood-effect site equilibration half-life is about 5 min), with consequent high brain concentrations and almost immediate attachment to the mu-receptor [6].

Reported time to detect change in breath interval after dose of 0.75 $\mu g \cdot kg^{-1}$ fentanyl was 0.9 s (SD 0.6) and time to peak effect 5.2 min (SD 1.4) [7]. The time to peak ventilatory depression effect after a 90 s infusion of fentanyl was 4.8 min (SD 2.2) irrespective of dose from 1.1 to 7.1 $\mu g \cdot kg^{-1}$ [6].

This quick action of fentanyl enabled the study to be completed in a short time (5 min after administration) with continual physiological monitoring and anaesthetist presence throughout. Furthermore, the quick effect enabled the

study to be performed with minimal disruption to the smooth running operating theatre schedule.

7.3.2 Fentanyl dose

Fentanyl passes the blood-brain barrier easily, so the peak effect occurs soon after administration. Intravenous boluses of fentanyl (1-3 μg·kg^{-1}) can produce potent and short-lasting analgesia (Table 7-1).

Variability between subjects response to opioids is 3 to 5-fold [3]. It is known that with high fentanyl doses (10-100 μg·kg^{-1}, over 10 times that needed to produce apnoea and profound analgesia in most individuals), the intensity of opioid effect (suppression of haemodynamic and hormonal responses to surgical stimulation) is not related to dose [454].

Table 7-1 Fentanyl dose range for clinical use

Use or outcome	Plasma concentration ng·mL^{-1}	Dose μg·kg^{-1}	70 kg dose μg
Analgesia [3, 6]	1-2		
Postoperative analgesia without respiratory depressant effects [6, 455]	1.5		
Intravenous bolus, potent and short-lasting analgesia		1-3	70-210
Infusion rates [6]		0.01-0.05 μg·kg^{-1}·min^{-1}	
Surgery [3, 6, 455]	2-3	2-5	140-350
Respiratory depression [6, 455, 456]	>2-3		
Anaesthetic induction loading dose (with a sedative-hypnotic, and a muscle relaxant) [3]		2-6	140-420
Major surgery [3, 6]	10		
High-dose opioid anaesthesia, rapid or slow bolus injections [3]	10-30	5-75	>350

Note: Adult plasma volume 2700-3000 ml

Fentanyl doses for this study were in the low range for anaesthetic induction loading dose: 50, 75, 100 and 150 μg (Figure 7-1).

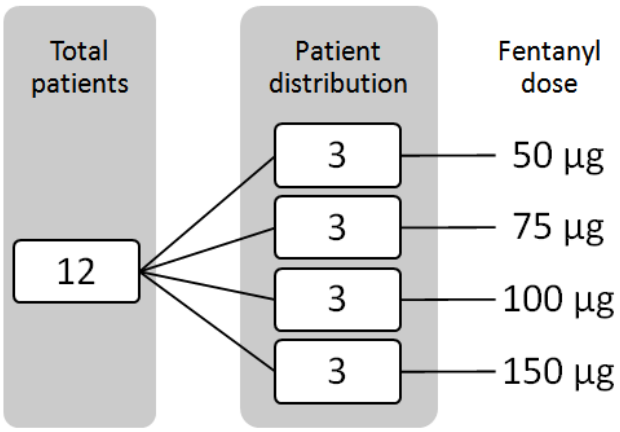


Figure 7-1 Patient distribution for fentanyl dose, numbers in boxes represent the number of patients in each group.

7.3.3 Fentanyl and midazolam

Although the purpose of this study was to explore the opioid fentanyl, pre-operatively midazolam is often given with fentanyl as they produce better sedation, analgesia and amnesia than do either drug alone [8]. Midazolam is a depressant of the central nervous system that can exacerbate the adverse effects of opioids: excessive sedation and respiratory depression [453] and should enhance the effect we are looking for. If midazolam was not used, higher doses of fentanyl would be required to ensure respiratory depression occurred within the study period.

All subjects received the same bolus dose of midazolam: 2.5 mg.

7.4 Subjects

Ethics approval for the study was given by the Southern Adelaide Flinders Clinical Human Research Ethics Committee (documentation Appendix D). Healthy subjects were recruited with no underlying cardiac, respiratory or autonomic nervous system problems such as diabetes. Patients were recruited if their anaesthetic plan included midazolam and fentanyl, a combination of sedatives commonly given pre-operatively to relax the patient before full anaesthesia. The subjects were recruited on a continual basis as suitable candidates arose in gynaecology, plastic surgery and general surgery.

7.4.1 Number of subjects

For this pilot observational study with unknown effect or effect size for the HRV indices being investigated, the number of participants was limited to 12.

7.4.2 Inclusion criteria

Subjects were eligible for inclusion if they were male or female, aged over 18 years, understood the explanation of the study in English, and were scheduled to receive pre-operative midazolam and fentanyl, with or without ventilation.

7.4.3 Exclusion criteria

Subjects were excluded if they had a high frequency of ventricular arrhythmias (premature complexes > 10 hr^{-1}), history of cardiac-rate controlling drugs, clinical signs of peripheral neuropathies or if they met any of the criteria listed in Table 7-2.

Table 7-2 Exclusion criteria for fentanyl study

Aged less than 18 or over 80	Unable to give consent
Allergy to fentanyl or midazolam	Pacemaker
Weight less than 40kg or more than 120kg (unsuitable for standard pre-medication dose of 2.5mg midazolam)	Requirement for administration of asthma medication pre-operatively
Previous severe nausea or vomiting associated with fentanyl administration	Administration of vagolytic or cardiac-rate controlling drugs in previous 48 hr
Regular use of opioid (e.g. daily Panadeine)	Heart transplant or myocardial infarct
Longstanding diabetes (> 2 years)	Previous history of >5% arrhythmias
If the investigation scheme was unsuitable for any other reason	

7.5 Procedure

Subjects were studied in the anaesthetic preparation room (Figure 7-2) for 10-15 min before surgical anaesthesia was induced. Before monitoring they had been supine for at least 15 min. Baseline physiological parameters were recorded for 5 min then a standard dose of the normal premedicant midazolam (2.5 mg) was administered followed by a randomly selected bolus of fentanyl

(50, 75, 100 or 150 µg). These were randomised by the statistician; three of each dose in numbered envelopes that were not opened until the subject was in the preparation room and ready to have baseline values recorded.

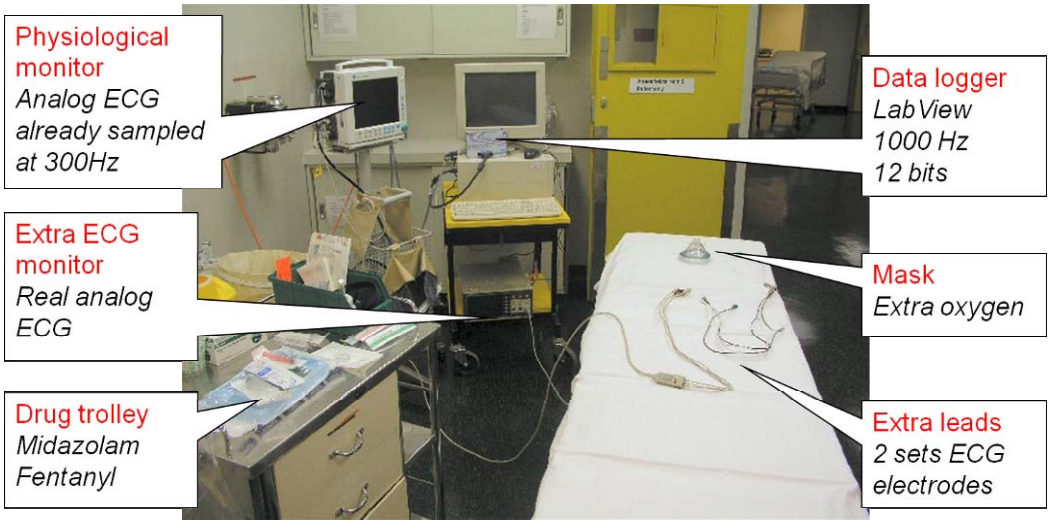


Figure 7-2 Layout of anaesthetic preparation room

Physiological parameters were recorded for a further 5 min while monitoring airway and cardiac parameters, following which propofol was administered with additional fentanyl as required for the operation to proceed.

7.5.1 Pillow

Patients were supine with their head on a pillow. Posture can affect the discharge pattern of muscles in the upper airway with tonic activity of the genioglossus increased with cervical flexion of the head [457].

7.5.2 Oxygen

Normally loss of airway tone with sedatives is created in anaesthesia to permit airway intervention with intubation.

This study required minimal intervention to the respiratory system and the airway, however subject safety had to be maintained. To meet both these requirements, oxygen was delivered to the subject through a facemask in anticipation of airway collapse to ensure full oxygenation before any respiratory depression occurred.

Oxygen and facemasks both affect HRV [248, 458] so these were applied from the start of the study for the full baseline period and continued for the duration of the study with intervention at the anaesthetist's discretion.

Timed breathing was not used for the baseline, and intubation did not proceed until the end of the study.

7.5.3 Noise

The anaesthetic preparation room adjoining the operating theatre was noisier than anticipated with people passing through, clinicians wanting to speak to the patient, monitoring equipment beeps and alarms. These environmental factors can impact sympathetic activity [459, 460] so, after the first subject, strict arrangements were made to prevent access to the room for the duration of the study and keep noise within the room to a minimum. The monitor beep and alarm volumes were turned down and the patient was offered earplugs though most refused.

7.6 Data Collection

7.6.1 Physiological data

The standard monitoring equipment in the operating theatre (Datex-Ohmeda AS/3 Anaesthetic Monitor, Instrumentarium Corporation, Finland, now sold by GE Healthcare, Chalfont St Giles, UK) was used to monitor physiological data: heart rate (HR), respiration rate from impedance across ECG leads (RRimped), oxygen saturation (SpO_2), respiration rate from airflow (RRspiro), and end-tidal carbon dioxide ($ETCO_2$, measured here in mmHg).

The data was recorded every 3 s with S/5 Collect software (GE Healthcare, Chalfont St Giles, UK), a 32-bit LabView application that recorded trend, waveform, event, and alarm data for off-line analysis.

7.6.2 Analog ECG

The Datex AS/3 is the normal physiological monitor used in the anaesthetic preparation room. Most data collection for HRV uses 1000 Hz sampling rate to capture the R-wave fiducial point to within 1 ms so the frequency response of the analog ECG output was tested with a white noise source (VHS Receiver

990R, Eddystone, Birmingham, UK). The results were recorded on a digital oscilloscope with FFT function (bandwidth 100 MHz, sampling rate[11] 1 GS·s^{-1}, TDS 2014, Tektronix, OR, USA).

It was found that the Datex AS/3 would not be suitable for HRV data as the analog ECG output is, in reality, pre-filtered at 300 Hz (Figure 7-3).

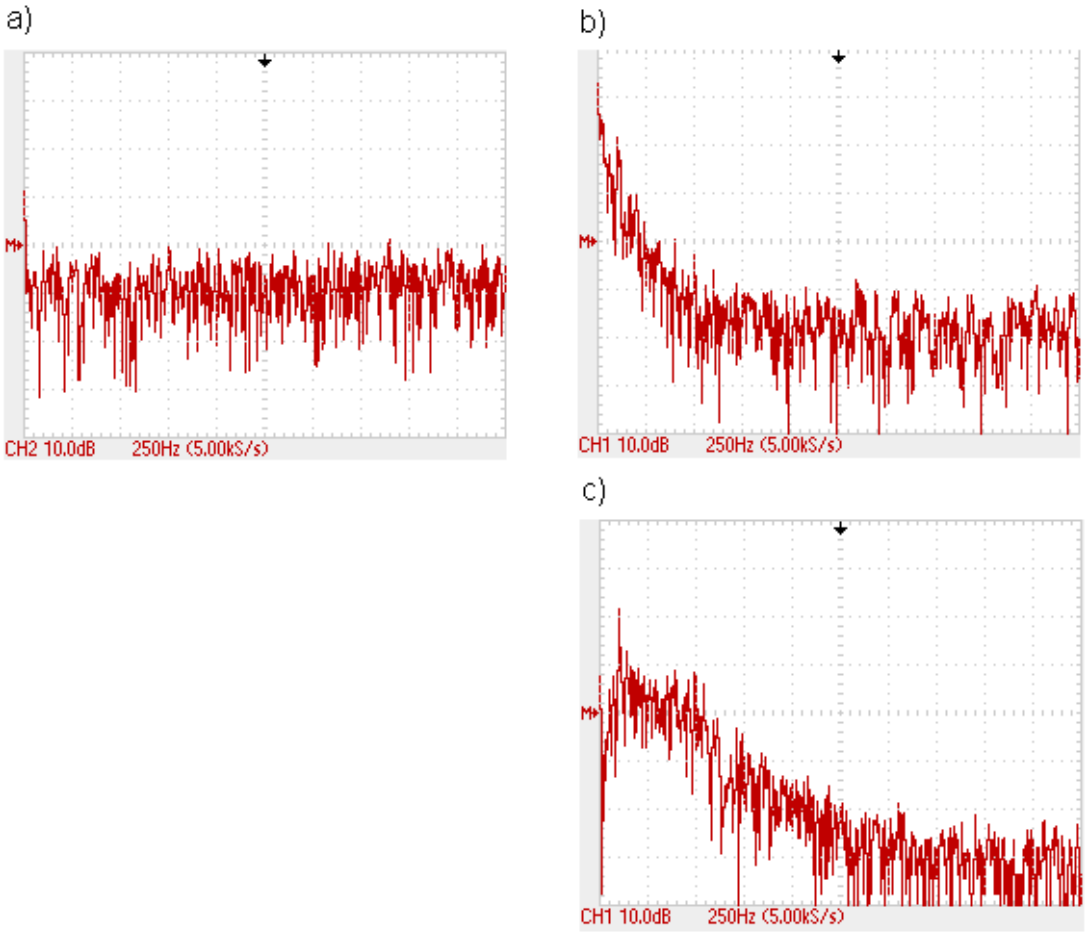


Figure 7-3 Frequency response of analog ECG monitors: a) white noise source with consistent frequency response from dc to 2.5 kHz, b) Datex AS/3 Anaesthetic Monitor with 30 dB fall in frequency response by 300 Hz, and c) HP 78353 Neonatal ECG Monitor has a slower roll-off with -30dB at 1000 Hz

Testing a variety of stand-alone ECG monitors found an HP device (Hewlett Packard 78353B, CA, USA) met the requirements for 1000 Hz sampling (Figure 7-3 c). This additional ECG monitor was used to collect the raw, unfiltered ECG from a second set of electrodes placed beside the anaesthetic monitor ECG electrodes in lead II configuration.

[11] GS.s^{-1} giga samples per second

The analog ECG from the HP device was digitised at 1000 Hz with 12 bits resolution (NI 6035E DAQ, National instruments Corp, TX, USA) and recorded with a LabView application (National Instruments Corp, TX, USA) for off-line analysis.

7.6.3 Clinical notes and data synchronisation

During the procedure, notes were taken on clinical observations relating to respiration, chest wall rigidity (known to occur with high dose fentanyl administration [453]) and also for deviations in the procedure.

Event buttons were pushed in quick succession on the Datex AS/3 (snapshot) and the analog ECG DAQ for synchronisation of the 2 separate data recordings at the following key events: start of study, mask adjustment, administration of fentanyl, apnoea noted by physiological monitor, clinical observation of upper airway collapse, end study.

7.7 Data processing

7.7.1 Analog ECG

R-wave detection

Analysis of the raw ECG data was performed using custom software developed for this study using MatLab (The MathWorks Inc., Natick, MA, USA). Detection of the fiducial point of the R-wave used two automatic methods in the first pass (Figure 7-4): simple threshold and multiple of backward differences [154, 461]. Visual inspection of the output of these two methods allowed selection of the method with the least artifacts. Manual alteration of the thresholds could further minimise the number of beats requiring manual correction. Signal conditioning with high pass filtering [462] did not impact the artifact rate so was not used.

Simple threshold, RRI Easy

A first pass threshold was set at one tenth of the maximum ECG value in the record. This could be manually altered in 10% jumps or with mouse selection for better discrimination of the R-wave depending on the overall recording characteristics for each subject.

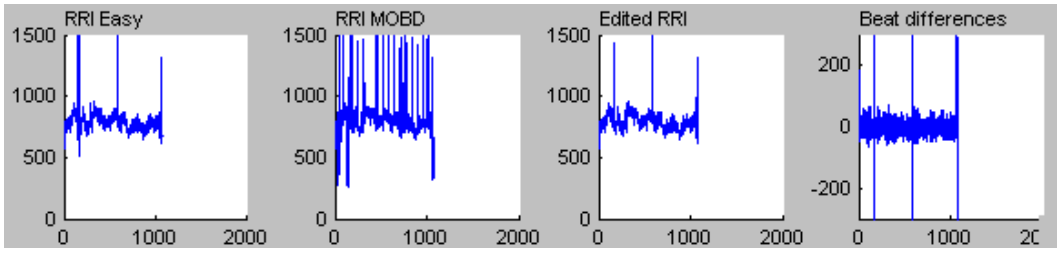


Figure 7-4 Results of first pass R-wave determination with missing or extra beats visible at twice or half the average RR-interval: RRI Easy uses a simple threshold; RRI MOBD uses the multiple of backward differences. Edited RRI shows where artifacts need manual correction and this is amplified in the last plot showing the differences between R-waves. For this subject, RRI Easy had the least number of artifacts requiring manual correction.

Multiple of backward differences, RRI MOBD

This method determines the energy of the QRS complex using the product of the absolute differences between four beats. The slope alone has been proven not sufficient to detect R-waves particularly for those with large amplitude and long duration [463]. Suppappola and Sun [464] showed this method using four beat differences required less processing time and had better accuracy than similar methods which use squaring of the backward differences or derivative estimates [465, 466] making it successful in real-time applications (although not a requirement for this study).

To reduce processing time, every second beat is investigated, the four backward differences are checked for sign consistency before determining the product, once the threshold is reached (initially set at 500M equivalent to 150^4 ADC units or 0.37 of typical R-wave peak[12]) the next 50 points (about 50 ms, a typical QRS complex being 100 ms) are inspected for the largest value which becomes the peak, the next 300 points are skipped.

R-wave manual correction

Artifacts were automatically identified if the RR-interval was more than 15% from the mean RR-interval (Figure 7-5). Also the longest and shortest seven intervals were identified for inspection. Flagged R-waves were visually verified and if necessary, corrected to the actual ECG R-wave peak.

[12] R-peak usually 1 mV, ECG output 1V/mV into 12 bits ADC (-5 to 5V), so a 1mV peak translates to 1Vo/p, or 410 ADC units

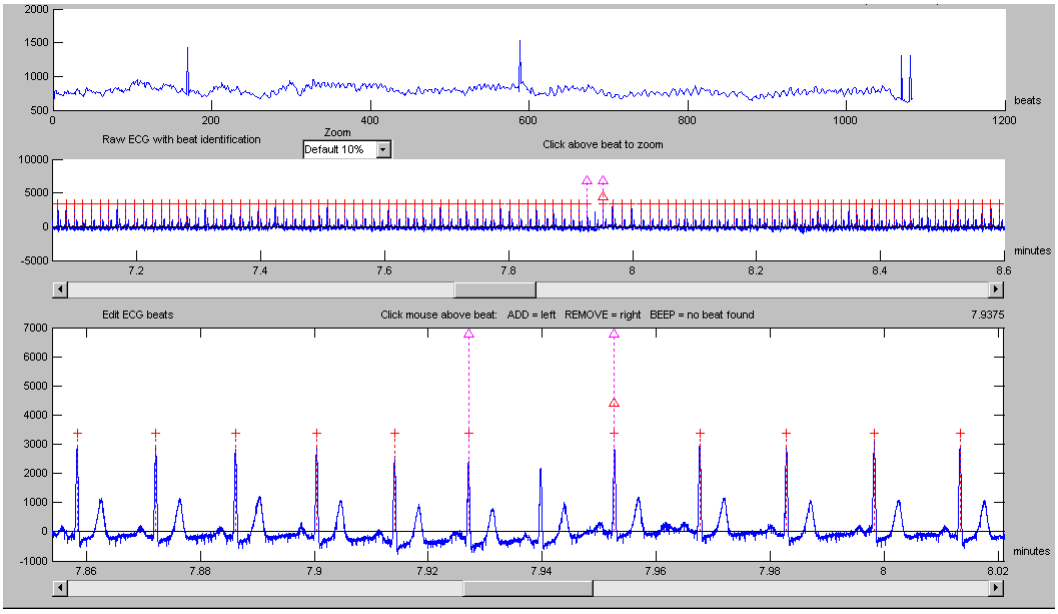


Figure 7-5 Manual editing of R-wave location with (top) full recording, (middle) 1.5 min of ECG, R-waves detected with cross, largest and smallest RR-intervals with triangles and (bottom) zoom to individual ECG beats with possible further zoom for exact placement of missing peaks

The few remaining artifacts were visually identified in a scan of the ECG and manually corrected. Correct identification of every R-wave was verified manually before analysis.

7.7.2 Physiological data

Numerical lists of physiological data ($ETCO_2$, RRspiro, SpO_2, HR, and RRimped) were plotted to allow identification and classification of the respiratory depression events.

The SpO_2 and $ETCO_2$ were quantified to determine the magnitude of hypoxia and hypercapnia through the different stages of the study: no mask (SpO_2 only), O_2 mask, after fentanyl administration before the initial respiratory depression event, and after respiratory depression.

Respiratory depression is variously defined as shallow respirations, irregular or periodic breathing and a respiratory rate $\leq$ 10 br·min^{-1} [4, 23, 26]. Low oxygen saturation, SpO_2 < 94%, is commonly used to determine hypoxia in respiratory depression, but with oxygen being continuously administered, a drop in saturation is unlikely to occur within the period of the study [26].

In this study clinically relevant respiratory depression was defined in consulatation with the anaesthetist as any of:

- $ETCO_2$ > 50 mmHg.
- An absolute $ETCO_2$ change from baseline >10 mmHg.
- Loss of $ETCO_2$ waveform.
- Cessation of ventilation: RRspiro = 0, or RRimped = 0.

These criteria were considered present if they occurred at any time during the procedure regardless of duration.

The physiological data was analysed for the occurrence of respiratory depression and the type of respiratory depression was classified using capnography, flow, and respiratory rate data:

- Central depression of respiratory centre; $ETCO_2$ change from baseline > 10 mmHg, but $ETCO_2$ >0.
- UAWO; upper airway obstructed if RRimped > 0 and $ETCO_2$ = 0 or RRspiro = 0.
- Critical respiratory depression event; no flow (RRspiro = 0 or $ETCO_2$ = 0) and RRimped unknown or RRimped = 0 (central apnoea).

Both the first respiratory depression event, and the first UAWO, were identified and cross-referenced (using interpolation between event marker positions) to the beat location within the RR-interval record.

The Datex monitor provided $ETCO_2$ in % and also provided ambient pressure to allow conversion to mmHg, the units used in this study.

7.8 Data analysis

7.8.1 HRV indices

The set of indices useful for very short-term HRV analysis over 30-beat periods were identified in Chapter 4.

It was determined in Chapter 5 that a window as short as 30-beats could still provide useful spectral density measures so this window size was selected as a trade-off between a small window that captures dynamic changes, the ability to generate meaningful indices from the number of beats, and the need to constrain the analysis to the period after fentanyl administration and before respiratory depression.

7.8.2 Analysis strategies

Four statistical analyses of the data were performed: a) effect of oxygen mask, b) post-fentanyl administration, post-F, c) peri-central depression, peri-CD, and d) peri-UAWO.

The baseline for these four analyses used the average of consecutive, non-overlapping 7x30-beat segments after the oxygen mask w concluding 30-beats before fentanyl administration where movement of the anaesthetist was likely to affect the subject's awareness and thus the HRV (Figure 7-6).

The effect of the oxygen mask compared the baseline to physiological data for Resting in Table 6-8. These baseline effects may hide or inflate fentanyl effects.

Post-fentanyl analysis compared the baseline to 10x30-beat segments starting with fentanyl administration.

Peri-CD analysis focused on the period preceding the initial central depression event with 10x30-beat segments (7 before and 2 after central depression) aligned about the central depression event.

Peri-UAWO analysis aligned the initial UAWO event, again with 10x30-beat segments (7 before and 2 after UAWO).

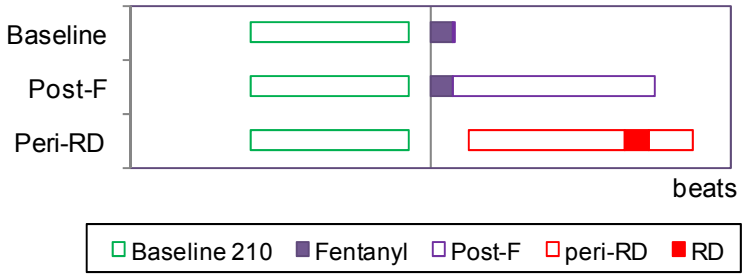


Figure 7-6 Analysis periods relative to fentanyl administration and respiratory depression event (RD)

One further analysis (non-statistical) looked at the individual subject responses for consecutive data from 240 beats before fentanyl to 350 beats after fentanyl. Indices selected for this investigation were: traditional time domain, spectral, and those identified as significant from the previous peri-RD analyses.

7.9 Statistical considerations

7.9.1 Confidence intervals

Confidence intervals for the 10 subjects were developed for the baseline and the subsequent 10 periods of interest for post-fentanyl and peri-RD analyses. Bootstrapped 95% confidence intervals of the median [225] were used for the analysis in MatLab with 10,000 replications required for confidence intervals [385]. The median is considered a more valid definition of centre with skewed frequency distributions because its accuracy is less influenced by variations in distributional shape [386]. The biased-corrected and accelerated percentile method was used for its qualities: transform respecting and second order accuracy [387] making it best for dealing with skew and bias in the bootstrap sampling distribution [386]. The bias-corrected and accelerated method can fail when the jackknife is performed on non-smooth data (e.g. median) and returns undefined results [387]. For these indices (indicated by ^ after index name), the corrected percentile method was used as it does use the jackknife [389].

7.9.2 Statistical significance

Overlap of the 95% confidence intervals were used to identify indices that showed significant differences between the baseline and time points post-fentanyl or peri-RD: $p<0.006$ for abutting, not overlapping confidence intervals,

$p<0.04$ with <50% overlap at (for small samples, n = 10, and difference in confidence interval width <2) [390, 391].

7.9.3 Correction for multiple tests

For this study, as in testing short-term indices (Chapter 6), the false discovery method was used to correct for multiple tests. With 31 indices, 10 comparisons (baseline to 10 post-fentanyl windows) and an alpha of 0.05, the corrected alpha ranged from 0.000016 to 0.05 (rank * 0.05/310).

Should any indices of HRV show significant association with loss of airway tone in this pilot study, the size of the effect from this study would inform subsequent confirmatory studies.

7.9.4 Significance of endpoint

Two events were selected for comparison: administration of fentanyl was used as a sham event to compare with the real event of respiratory depression. The baseline was different for each event and began a constant time before the event; the time selected so both event baselines were in the pre-fentanyl period. The change from baselines for the region immediately preceding sham and real events were compared using a paired Student's t-test.

The ability to perform statistical analysis on the difference from the baseline depends on the number of subjects that reach the same respiratory depression endpoint, and the time available for the baselines.

7.9.5 Sample size and power

This substantially descriptive study needed enough subjects to provide a cross-section of patient types with a range of different responses and that was realistic in the time frame available.

There has been no attempt to satisfy statistical power requirements; the variance of the selected indices over short time periods in response to fentanyl is unknown.

7.10 Summary

The study was designed to minimise changes to patient management in the pre-operative setting and ensure subject safety with full physiological monitoring and anaesthetist attendance.

The use of fentanyl allowed the study to be performed pre-operatively within a short time frame.

Chapter 8. Results for HRV of fentanyl-induced loss of airway tone

8.1 Introduction

The analog unfiltered ECG and the physiological data: HR, $ETCO_2$, SpO_2, RRspiro and RRimped; were collected and processed using a custom application developed in MatLab to determine if changes in HRV preceded a respiratory depression event.

8.2 Procedure details

8.2.1 Subject enrolment

Data was collected for 12 of the 15 consenting subjects enrolled in this trial. Problems in the operating theatre prevented three enrolled subjects from starting the trial (one subject not admitted by surgeon into theatre early enough for the study, and two operations cancelled due to timing constraints unrelated to this study).

Two sets of data were excluded due to poor mask application over the baseline period leaving 10 sets of data for analysis. Full subject exclusion details are provided in Figure 8-1.

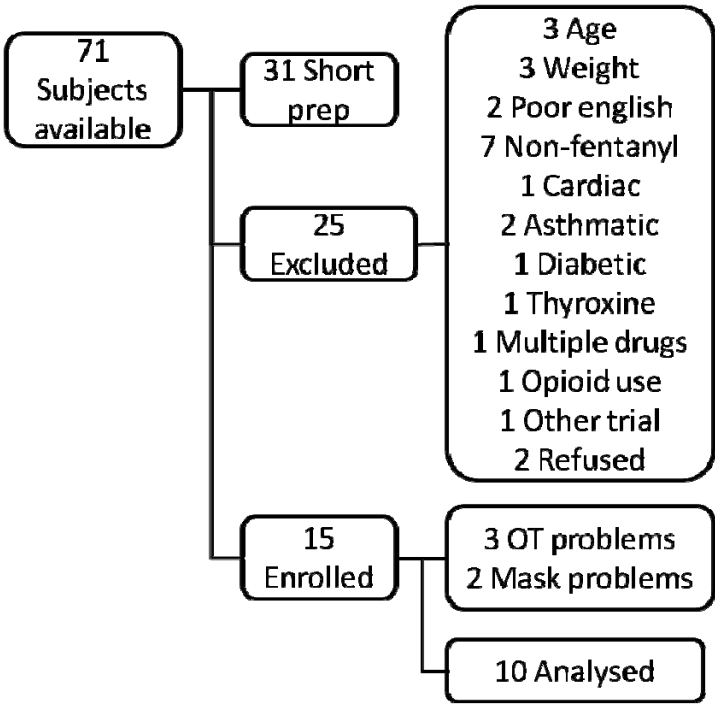


Figure 8-1 Subject enrolment flowchart Notes: Short prep, not enough time to explain the study and get consent while nursing preparation occurred; Refused, not interested and previous bad experience with facemask.

Female subjects (N = 8/10) predominated in the scheduled operating lists available for this study (Table 8-1).

Table 8-1 Summary of patient data

	F N = 8 Mean (SD)	M N = 2 Mean (SD)	Total N = 10 Mean (SD)
Age	42.8 (14.4)	36.0 (25.5)	41.1 (15.5)
Weight	76.0 (12.1)	93.0 (5.7)	79.4 (13.0)

8.2.2 Fentanyl and midazolam dose

The mean fentanyl dose was 1.2 μg·kg^{-1} and the mean midazolam dose was 0.032 mg·kg^{-1} (Table 8-2).

8.2.3 Procedure timing

The study periods ranged from 11 to 40 min (Table 8-2). Although the intention was to record 5 min before and 5 min after fentanyl administration, some studies went for longer.

Table 8-2 Subject details: age, fentanyl and midazolam dose, and procedure time

		Fentanyl		Midazolam		Procedure
Subject	Age	μg	μg·kg^{-1}	mg	mg·kg^{-1}	min
1	54	100	1.0	2.5	0.026	11:35
2	42	75	0.9	2.5	0.029	13:32
3	35	50	0.6	2.5	0.029	13:45
4	46	50	0.8	2.5	0.038	15:20
5	38	150	1.5	2.5	0.026	25:26
6	18	75	0.8	2.5	0.028	14.01
7	68	50	0.8	2.5	0.039	25:25
8	24	150	2.1	2.5	0.035	12:35
9	31	150	2.0	2.5	0.034	14:12
10	58	100	1.6	2.5	0.039	39:41
Mean (SD)	41		1.2 (0.55)		0.032 (0.005)	18:30 (9)

Note: The other doses of 75 and 100 μg fentanyl were given to the two subjects excluded for baseline mask problems

Extended baselines were recorded when the anaesthetist was still monitoring the preceding patient while the study had started. After the study, monitoring continued until the operating theatre was cleared and ready for the subject's operation to proceed.

8.3 Data processing

8.3.1 ECG

The raw ECG from the HP monitor was generally of good to excellent quality. A summary of the ECG quality with comments on pre-processing actions taken is provided in Table 8-3. Two subjects had baseline wander, and one subject had an R-wave that sometimes approached the same height as the T-wave which caused some missed beats that needed manual correction. There were no artifacts caused by premature ventricular beats or ectopic beats.

Table 8-3 ECG quality and processing comments

Subject	ECG quality	Beats>15%	Comment
1	Excellent	None	
2	Excellent	3 OK	
3	Excellent, some baseline movement	None	Large RR-interval changes in final 30 s, not PVC, final 30 s excluded
4	Excellent	4 OK	
5	Good	None	R-wave negative, used inverted ECG
6	Excellent	39 OK	
7	Good, some baseline movement	3 OK	2 possible R-wave locations altered
8	Noisy, R-wave often same height as T wave	52 OK	Beat position guessed at 1.34, 1.66, 3.14 and 3.16 min
9	Excellent	6 OK	10 changes made to R-wave location in area with shifting baseline
10	Excellent	None	

Note: OK indicates visual inspection of beats >15% different from mean RR-interval were normal sinus beats

The RR-interval data record for each subject was kept complete from the start to the end of the study. A visual inspection of the RR-interval tachograms (Figure 8-2) confirmed no missed beats or double counted beats (i.e. visible at twice or half the mean RR-interval).

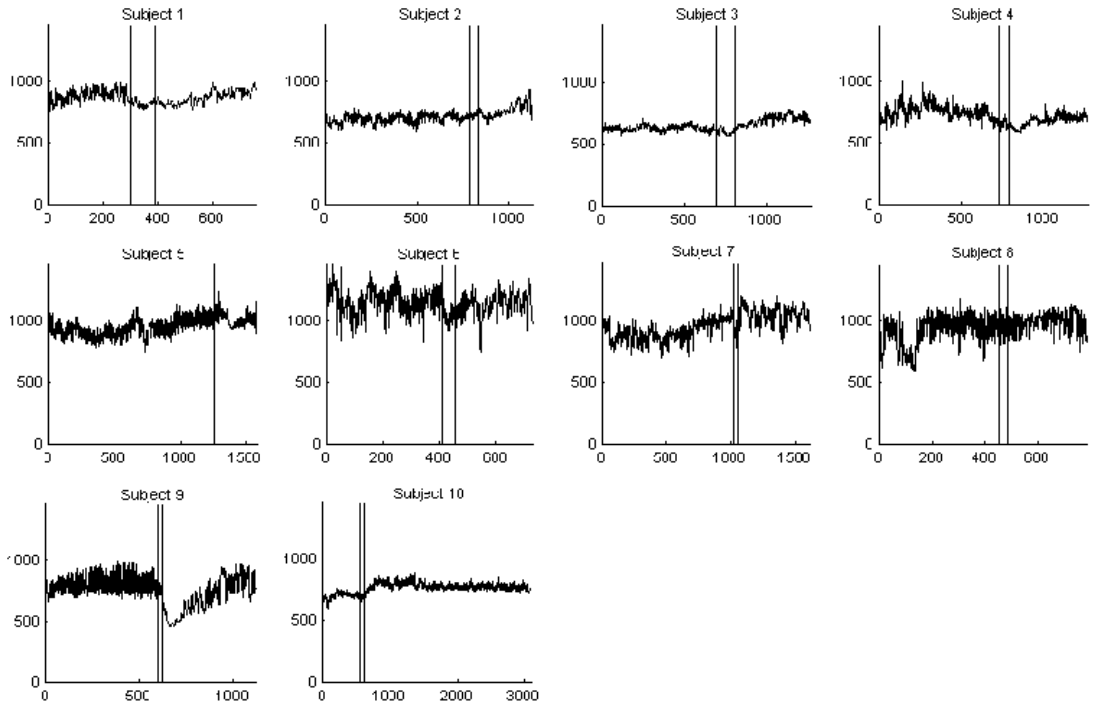


Figure 8-2 RR-interval tachogram for each subject (x-axis is beats). The two vertical lines enclose fentanyl administration.

Observations of the RR-interval tachogram showed the response following fentanyl administration was not consistent for all subjects:

- Decreased RR-interval in subjects 5, 6 and 9.
- Increased RR-interval in subjects 2, 3 and 10.
- Biphasic response in subject 7.
- Decreased variability in subjects 1, 4 and a small decrease in 8.

There is little evidence of bradycardia, heart rate < 60bpm, which would show on these plots as RR-interval > 1000. (Note: Heart rate in bpm is 60,000/RR-interval in ms).

8.3.2 SpO_2 and $ETCO_2$

The physiological data was analysed to provide the mean and range of SpO_2 and $ETCO_2$ to determine if hypercapnia or hypoxia occurred during the study.

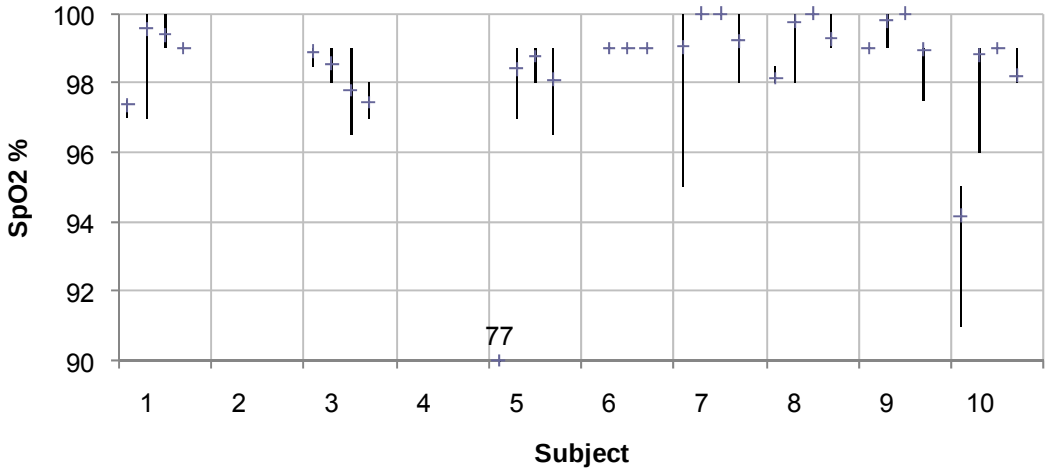


Figure 8-3 SpO_2 (mean and range) for each subject during 4 stages of the study: a) no mask, b) O_2 mask, c) from fentanyl administration to initial CD, and d) post-RD.

The SpO_2 was not recorded for 2 subjects. Pre-mask levels of SpO_2 were low in subjects 5 and 10, with minimums of 77% and 91%. It is likely the subject 5 pre-mask data was an artifact as it was in this range for 3 min with no clinical note that the subject was blue. For the eight of ten subjects with data, the SpO_2 remained above 96% throughout the study once the O_2 mask was in place.

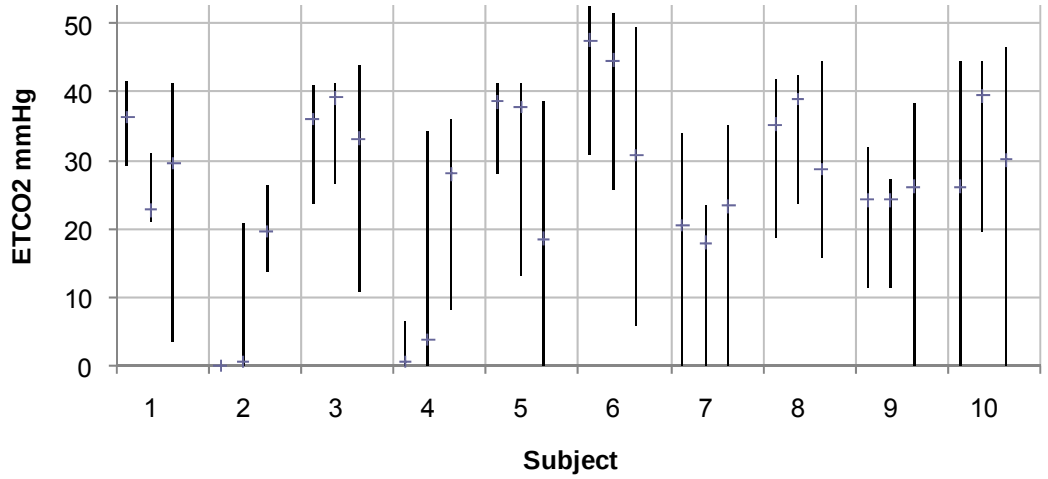


Figure 8-4 $ETCO_2$ (mean and range) for each subject during 3 stages of the study: a) O_2 mask, b) from fentanyl administration to initial CD, and c) post-RD

The maximum $ETCO_2$ after respiratory depression tended to be slightly above the baseline maximum. Two subjects, 6 and 10, had high levels even before fentanyl with maximum $ETCO_2$ of 53 and 44 mmHg. Hyperventilation was not

present and there was no clinical explanation[13] for these high values. Hypercapnia, $ETCO_2$ > 45 mmHg, occurred only toward the end of the study.

8.3.3 Respiratory depression

Clinical signs

Three subjects reached the endpoint of complete loss of upper airway tone confirmed by the anaesthetist, two subjects had partial collapse indicated by snoring, and three more subjects had short duration (20 s) apnoea detected by the monitoring equipment (Table 8-4). No chest wall rigidity was detected at any time. Also noted were mask repositioning during baseline (subjects 1, 2 and 7) and noises after fentanyl administration that caused a startle reflex (subjects 1, 6, 8 and 10).

The three subjects with complete loss of upper airway tone were also those who had the lowest baseline levels of SpO_2 before the O_2 mask was used.

Physiological data

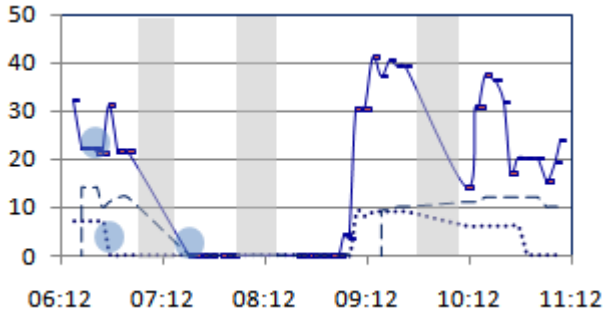
Subsequent analysis of physiological data identified the CD and UAWO events for each subject with capnography, spirometry, and chest impedance (Figure 8-5 to 8-7).

The development of respiratory depression was different for individuals, and the type of respiratory depression changed with time. In general, fentanyl administration was followed by CD; in some subjects this was directly followed with UAWO (7 and 9) others had multiple CD events (3, 6 and 8). In two subjects the CD occurred simultaneously with flow of zero (4 and 5). These concurrent events have been separated by one beat to enable plots to show both event lines.

Critical respiratory events were identified when UAWO could not be confirmed by RRimped > 0 due to: missing data (subjects 2, 4, and 6); noisy signal (subjects 8 and 9); or central apnoea (no occurrences before initial respiratory events where $ETCO_2$ = 0 or RRspiro = 0).

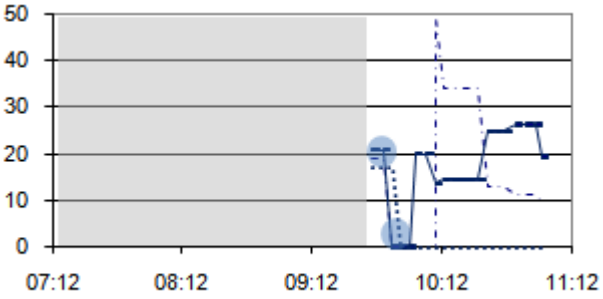
[13] $ETCO_2$ increases with hyperventilation which can be caused by altered mental status such as overdose, sedation, intoxication, head trauma, or stroke, or by a tiring patient with congestive heart failure. Other reasons CO2 may be high: Increased cardiac output with increased breathing, fever, sepsis, pain, severe difficulty breathing, depressed respirations, chronic hypercapnia.

a) Subject 1



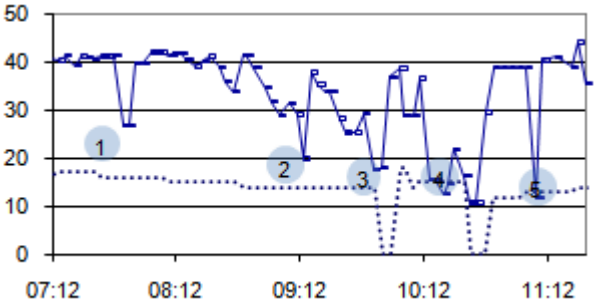
Fast descent into central depression, with UAWO (RRspiro = 0 and RRimped > 0) soon after. Missing data until $ETCO_2$ = 0. No clinical observation of UAWO.

b) Subject 2



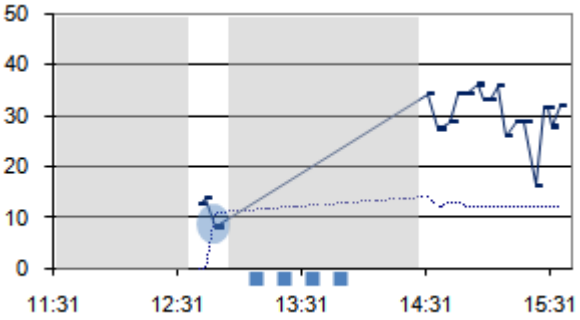
Long missing data block immediately after fentanyl. First recorded CD followed closely by $ETCO_2$ = 0 and RRspiro = 0. No UAWO seen by observation. RRimped missing, critical respiratory event.

c) Subject 3



Five separate CD events, with third associated with RRspiro = 0. RRimped missing, critical respiratory event.
No UAWO by capnography or clinical observation.

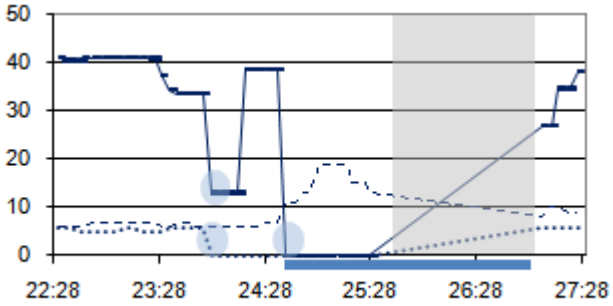
d) Subject 4



CD with RRspiro=0 seen between missing data blocks. RRimped missing, critical respiratory event. UAWO soon after with snoring.

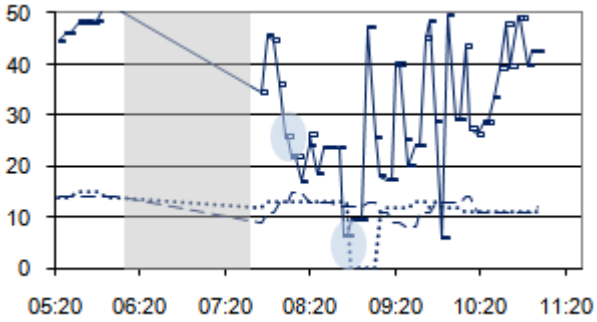
Figure 8-5 a)-d) Subjects 1-4 identification of respiratory depression events Legend: $ETCO_2$ solid line, RRspiro dotted, RRimped dashed, RR_{CO2} dash-dot line, shaded block is missing data, clinically observed UAWO in bar below x-axis: full (solid line) and partial (broken line)

a) Subject 5



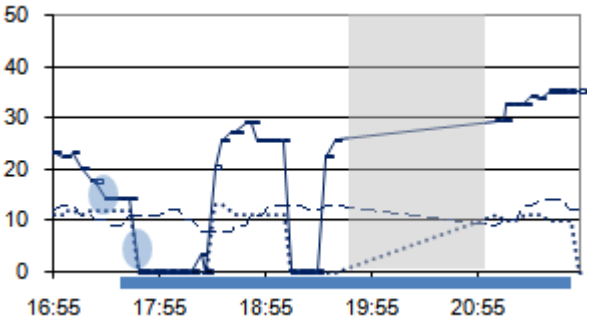
Descent into CD accompanied by UAWO (RRspiro=0 and RRimped > 0). Drop of $ETCO_2$ =0 occurs later with long clinical UAWO.

b) Subject 6



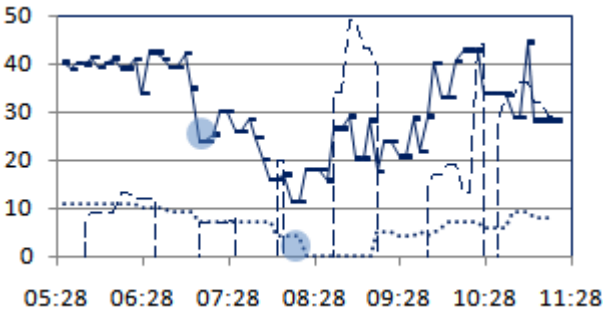
Missing data before first CD event. Delay until UAWO (RRspiro = 0 and RRimped > 0). No events where $ETCO_2$ = 0.

c) Subject 7



Short CD followed by UAWO ($ETCO_2$ = 0, RRspiro = 0, RRimped > 0) and clinical observation of sustained UAWO.

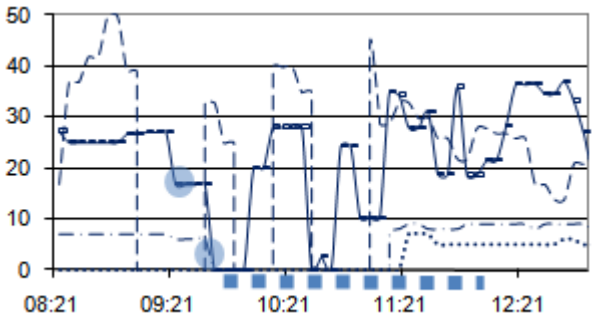
d) Subject 8



Slow descent into CD, with delay until RRspiro = 0, RRimped noisy, critical respiratory event. $ETCO_2$ remained > 0. No clinical sign of UAWO.

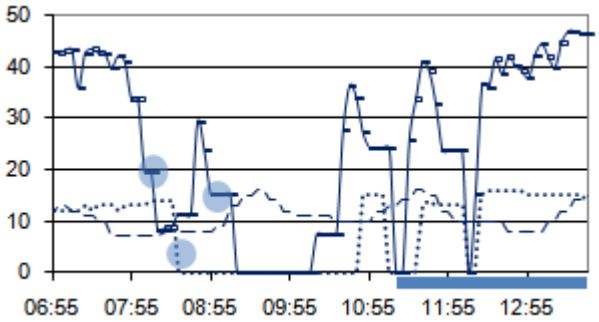
Figure 8-6 a)-d) Subjects 5-8 identification of respiratory depression events Explanation as for Figure 8-5

a) Subject 9



Short CD followed by UAWO ($ETCO_2$ = 0, RRimped noisy), critical respiratory event with UAWO (snoring) observed after this. RRspiro not working for part - no flow event could have occurred earlier. Note: Although RRimped fluctuates wildly, RR_{CO2} shows no hyperventilation

b) Subject 10



Descent from CD into UAWO (RRspiro = 0 and RRimped > 0), much earlier than $ETCO_2$ = 0 or the clinical observation of UAWO.

Figure 8-7 a)-b) Subjects 9-10 identification of respiratory depression events Explanation as for Figure 8-5

The descent to respiratory depression is summarised in Table 8-4 with events aligned on the occurrence of upper airway obstruction ($ETCO_2$ = 0). A more detailed description of event chronology is provided in Table 8-5.

Table 8-4 Summary of respiratory depression events for each subject

Subject	Initial respiratory events				Clinical observation
1			C	U_F	Apnoea
2		M	C	F	Possible RD
3	C	C	C	F	None
4			M	C/F	Snoring UAWO
5				C/U_F	UAWO
6	M	C	C	U_F	Shallow respiration
7			C	U_{EF}	UAWO
8		C	C	F	Shallow respiration
9			C	E	Snoring UAWO
10			C	U_F	UAWO

Abbreviations: C, central depression; F, loss of flow with RRspiro = 0; M, missing data; E, $ETCO_2$ = 0; U_E, upper airway obstruction with $ETCO_2$ = 0 confirmed by RRimped = 0; U_F, upper airway obstruction with RRspiro = 0 confirmed by RRimped = 0.

Table 8-5 Details of key study events for subjects

Subj	Event log and clinical notes	Physiological signs	RD type	Datex min	Beat number
1	Mask on			0:30	11
	Mask to assistant			4:46	299
	Fentanyl			5:20	340
		$ETCO_2$ 22	CD	6:24	416*
	Door close, startle			6:36	427
		RRspiro 0		6:40	435**
		$ETCO_2$ 0	UAWO	7:27	488
	Ev4 apnoea or mask slip	$ETCO_2$ 0	Clin	7:54	527
2	Mask on			3:45	520
	Fentanyl			6:57	788
		Missing data	?	6:57-9:45	788-981
		$ETCO_2$ 20	CD	9:45	981*
		$ETCO_2$ 0	CRE	9:49	986**
		RRspiro 0		9:53	992
	Ev7 Possible RD		Clin		1018
3	Mask on			0:00	221
	Fentanyl			5:13	696
		$ETCO_2$ 27	CD	7:46	928*
		$ETCO_2$ 20	CD	9:14	1065
		$ETCO_2$ 18	CD	9:48	1117
		RRspiro 0	CRE	9:52	1124**
		$ETCO_2$ 10, RRspiro 0	CD	10:35	1190
		$ETCO_2$ 11	CD	11:05	1237
4	Mask on			6:43	390
	Fentanyl			10:00	726
		Missing data	?	10:30-12:42	788-985
		$ETCO_2$ 13, RRspiro 0	CD, CRE	12:42	985*,**
		$ETCO_2$ 8	CD	12:50	997
	Ev6 snoring		Clin	13:12	1025
		Missing data	?	12:50-14:32	997-1144
5	Mask on			17:18	961
	Fentanyl			22:31	1255
		$ETCO_2$ 13, RRspiro 0	CD, UAWO	23:58	1343*,**
		$ETCO_2$ 0	UAWO	24:40	1384
	Ev5-Ev7 no flow		Clin	24:38-26:43	1382-1508
6	Mask on			0:00	152
	Fentanyl			5:23	407
		Missing data	?	6:03-8:03	450-550
	Ev4 shallow breath	$ETCO_2$ 26	CD	8:03	550*
	Door open, startle	$ETCO_2$ 17	CD	8:14	559

Subj	Event log and clinical notes	Physiological signs	RD type	Datex min	Beat number
		$ETCO_2$ 7	CD	8:43	584
6		RRspiro 0	UAWO	8:49	589**
	Ev5 RR almost 0			9:10	602
7	Mask resited			10:27	712
	Fentanyl			16:56	1018
		$ETCO_2$ 14	CD	17:25	1095*
		$ETCO_2$ 0, RRspiro 0, RRimped OK	UAWO	17:44	1113**
	Ev6-12 UAWO		Clin	18:02-21:58	1141-1368
8	Mask on			00:37	138
	Fentanyl			5:32	453
		$ETCO_2$ 23	CD	7:07	542*
		$ETCO_2$ 15	CD	7:57	594
		$ETCO_2$ 12	CD	8:12	609
		RRspiro 0	CRE	8:22	620**
	Ev5 $ETCO_2$ near flat		ClinCD	8:27	629
	Ev6 pager, startle			8:57	660
9	Mask on			0:35	19
	Fentanyl			8:15	603
		$ETCO_2$ 17	CD	9:25	727*
		$ETCO_2$ 0, RRspiro 0	CRE	9:45	754**
	Ev5 snoring		UAWO	10:30	833
10	Mask on			1:45	137
	Fentanyl			6:50	567
		$ETCO_2$ 20	CD	8:05	663*
		$ETCO_2$ 9	CD	8:15	677
		RRspiro 0, RRimped OK	UAWO	8:30	697**
		$ETCO_2$ 0	UAWO	9:15	758
	Ev4 Datex apnoea		Clin	9:20	777
	Ev5 breathing			10:45	879
		RRspiro 0	UAWO	11:10	913
	Ev6-8 long obstruction	$ETCO_2$ 0	UAWO	11:15-12:50	916-1035
	Ev9 breathing			13:40	1093
	Ev11 crash in OT, startle			18:05	1428

Notes: * beat number for CD; ** beat number for UAWO.
Abbreviations: CD, central depression; $ETCO_2$, end-tidal carbon dioxide; Ev, event log number; RD, respiratory depression; RR, respiratory rate from a) CO_2, capnography, b) imped, ECG electrode chest impedance, and c) spiro, spirometry; SpO_2, oxygen saturation; UAWO, upper airway obstruction.

Problems analysing physiological data

Analysis of the physiological data proved difficult for some subjects:

- Missing Datex data collection due to computer buffering overflow errors. This problem was not observed until in-depth data analysis mid-way through the trial showed intermittent missing time intervals and the study was put on hold until this was corrected. This had an effect on identifying the timing of the initial respiratory depression event using spirometry and capnography parameters.
- Missing individual physiological data due to equipment problems. Impedance from ECG electrodes was not available (3 subjects) and was problematic (highly variable, noisy) in two subjects (8 and 9) so UAWO could not be confirmed. Spirometry was not recorded for the first part of subject 9 data so the initial no flow event could have occurred earlier.
- The expert clinicians took care to minimise mask leak with observations of breathing bag movement but if it occurred for > 15 s it could be interpreted as a critical respiratory event with $ETCO_2$ and RRspiro both zero.
- Baseline $ETCO_2$ was not normal for some individuals for no apparent reason:
  - low, <30 mmHg, for subject 9;
  - high, >45 mmHg, for subject 6 with no sign of hyperventilation;
  - with large variance, 15 to 40 mmHg, for subject 10.

8.3.4 Relative timing of events

The number of beats that occurred from the start of the study to key events, and during key periods (baseline, and from fentanyl administration to CD and UAWO) are given in Table 8-6.

The 210-beat baseline started 240 beats before fentanyl administration and excluded the last 30-beat period when the anaesthetist was active. This fit within the minimum baseline for subject 6 with 225 beats available from the time of oxygen mask application. The median time for fentanyl administration

(including midazolam and saline) was 49 beats (35 s). This dropped to 41 beats if the outlier (119 beats for subject 3) was excluded.

Table 8-6 Number of beats between key events: O_2 mask on, fentanyl administration, CD and UAWO

Subject	Number of beats from start of study					Number of beats for period			
	Mask	Start F	End F	CD	UAWO	Base-line	F	Start F to CD	Start F to UAWO
1	11	340	392	416	435	299	52	76	95
2	520	788	830	981	986	238	41	193	198
3	221	696	815	928	1124	445	119	232	428
4	390	726	788	985	986	306	62	259	260
5	961	1255	1285	1343	1344	264	29	88	89
6	152	407	451	550	589	225	43	143	182
7	712	1018	1058	1095	1113	276	38	77	95
8	138	453	484	542	620	285	31	89	167
9	19	603	623	727	754	554	20	124	151
10	137	567	619	663	697	400	52	96	130
Min						225	20	76	89
Mean						329	49	138	180
Max						554	119	259	428

Notes: Baseline is from mask to 30 beats pre-fentanyl.
Abbreviations: CD,central depression; F, fentanyl administration; UAWO, upper airway obstruction.

For each subject, separate lines in Figure 8-8 show the relative timing of the events: mask application before baseline, start of fentanyl administration and 270 beats following this, initial central depression and critical respiratory depression, and extent of observed clinical UAWO.

Post-fentanyl analysis was as planned from the start of fentanyl administration for 300 beats.

Peri-CD analysis used 210 beats preceding the identified respiratory depression event, and 90 beats after. The initial respiratory depression event seen in all subjects was CD ($ETCO_2$ >10mmHg from baseline). The time from fentanyl administration to the initial CD event ranged from 76 to 259 beats.

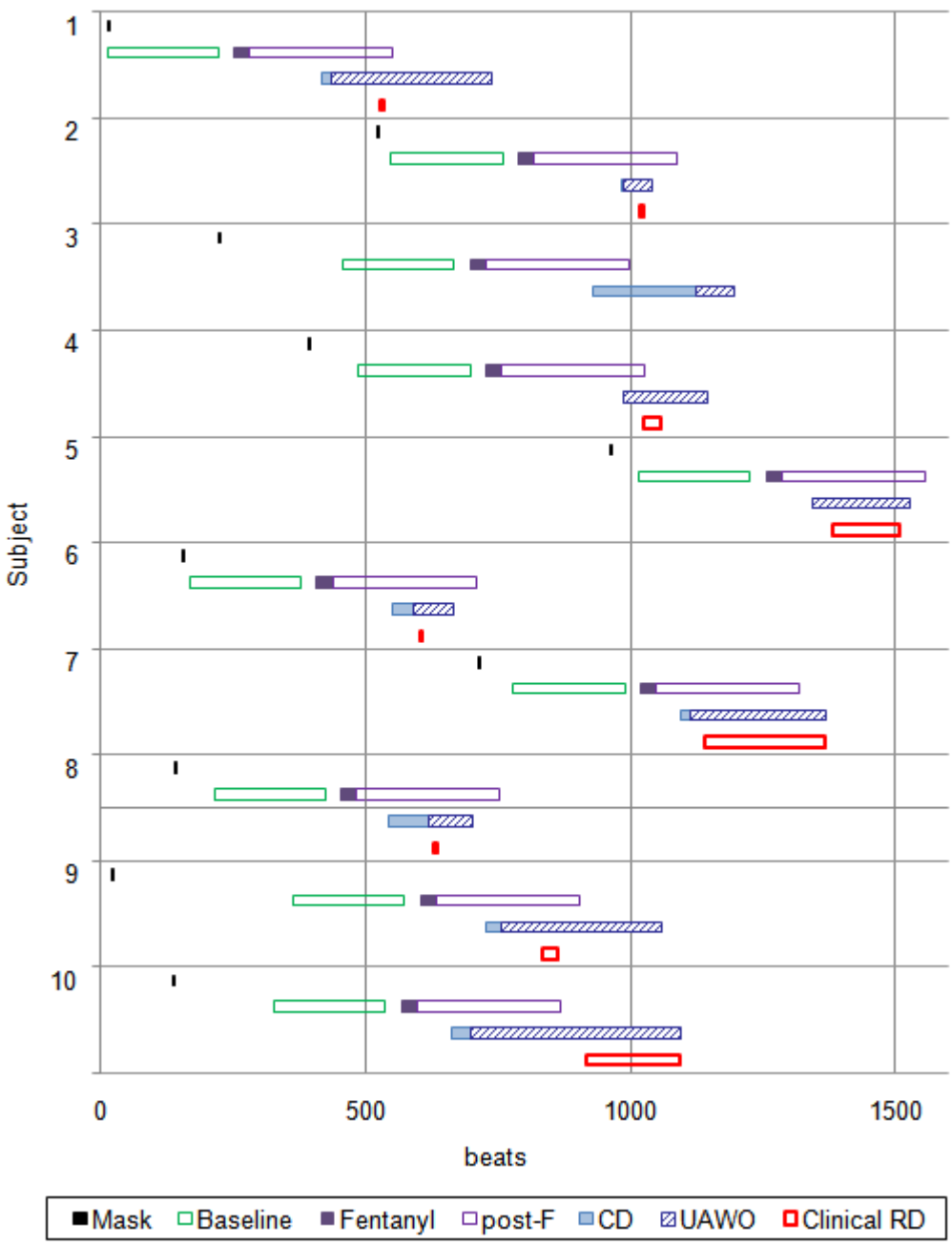


Figure 8-8 Relative timing of events for each subjectwith 4 bars showing: 1) mask application; 2) baseline 210 beats, fentanyl administration and 270 beats after fentanyl; 3) respiratory depression; and 4) extent of clinical observation (medium sized events indicate snoring, longer events are UAWO). Abbreviations: CD, first central depression event; Clinical RD, clinically observed respiratory depression; post-F, post-fentanyl; UAWO, upper airway obstruction.

Alignment of the initial CD events for analysis meant that the 210 beats preceding CD overlapped the baseline by variable amounts for most subjects.

Critical respiratory depression ($ETCO_2$ = 0 or RRspiro = 0) occurred in all subjects 89 to 428 beats (mean 180) after fentanyl administration. The time until clinically observed UAWO was slightly longer with a mean of 213 beats (range 123 to 349).

8.4 Effect of oxygen mask on HRV baseline

The O_2 mask baseline is given in the first column of confidence intervals in Table 8-7. Compared to Resting (Table 6-8), O_2 mask had a decreased **meanRR** (95% CI 757–957 from 965–988 ms), and increased total variability **LombTotal** and sympathovagal balance **SDNN/RMSSD** (# p<0.006).

The effect on HRV of the oxygen facemask can be seen in Figure 8-9 where it is compared to baseline confidence intervals for the different physiological states presented in Chapter 6. The O_2 mask is similar to Meditation with increased **LombTotal** and **pQc** (runs of decreasing RR-intervals), but is similar to Sleep in **SDNNmc** and **SDNN/RMSSD**. O_2 mask is different to both Sleep and Meditation (Figure 8-9 c) in **assym(R/L)**, **pQa**, **skewRRz**, and **TACI(20)**.

Table 8-7 Post-fentanyl 95% confidence intervals for median of indices for baseline and 10 consecutive 30-beat intervals from fentanyl administration (every third interval shown)

Index	O_2 mask Baseline[a]	*p1*	F3(c)	F6 (f)	F9 (i)	*p2*
1: SDNN	33.1–47.5		13.1–49.1	12.4–50.4	24.8–71.4	d'
2: SDNNmc	3.98–4.98		2.02–5.01	1.66–5.24	2.94–6.09	
3: RMSSD	22.6–39.2		8.53–54.56	9.21–29.30	13.0–47.0	d'
4: RMSSDmc	2.80–4.28		1.20–4.80	1.25–3.26	1.56–5.36	d',f'
5: pNN20 ^	34.5–55.2		1.72–51.72	1.72–44.83	10.3–55.2	d'
6: Lomb LF	20.3–33.2		14.8–31.5	18.9–37.3	19.5–32.5	
7: LombHF	12.1–26.1		12.6–26.2	11.4–23.8	9.17–24.17	
8: Lomb HFnu	26.1–52.3		31.7–55.9	25.6–56.7	19.9–56.1	
9: Lomb LF/HF	0.91–2.83		0.83–2.07	0.76–2.91	0.81–4.02	
10: LombTotal	49.6–52.0	#	39.5–51.6	32.5–52.7	35.9–51.1	b',e,g,h',j
11: accel	0.48–0.55		0.52–0.63	0.41–0.62	0.48–0.65	
12: acv0x	3.00–3.00		0.00–3.00	2.00–5.00	0.00–4.00	
13: assym(R/L)	0.80–1.07		0.45–3.08	0.71–2.73	0.20–1.92	
14: CVdRR	124–128		124–136	123–139	125–141	
15: gradRR	-0.55–1.17		-0.40–1.11	-1.07–3.72	-1.03–2.54	
16: kurtRRz	2.19–2.55		1.95–2.89	2.17–2.56	2.06–3.19	d'
17: mean.r(L1-6)	-0.03–0.05		-0.02–0.35	-0.00–0.35	0.10–0.34	d',e,g',h,i,j'
18: meanRR	757–947	#	622–1042	648–1035	672–1017	
19: norm.dRR	0.11–0.13		0.10–0.14	0.10–0.16	0.10–0.19	
20: normRR	0.11–0.13		0.11–0.19	0.10–0.15	0.12–0.19	h
21: PolVar20 ^	0.00–2.08		0.00–87.50	0.00–95.83	0.00–22.92	a',b',c',d', e',f',g',h',j'
22: pQa	15.1–18.5	#	17.9–27.0	18.1–25.5	15.1–25.0	c',e',f'
23: pQb	25.5–30.8	#	18.5–36.3	23.1–37.0	23.2–41.9	h'
24: pQc	29.6–35.7	#	18.5–32.0	22.3–32.1	21.4–33.9	d'
25: r(RR)	0.63–0.74	#	0.43–0.79	0.56–0.84	0.53–0.91	
26: SDNN/RMSSD	1.17–1.43	#	0.96–1.60	1.10–1.78	1.07–2.31	
27: skew.dRR	0.68–0.89		0.48–1.06	0.64–1.43	0.78–1.43	
28: skewRRz	-0.17–0.03		-0.23–0.60	-0.50–0.53	-0.71–0.20	g
29: sign(dRR) ^	-2.00–1.00		-2.50–3.00	-0.50–3.00	-3.00–4.00	
30: TACI(10)	0.20–0.33	#	0.13–0.51	0.00–0.60	0.20–0.39	d,e
31: TACI(20)	0.15–0.28	#	0.00–1.00	0.00–0.50	0.00–0.40	d'

Note: Correction for multiple comparisons removed (greyed) all but five significant results for four indices

[a] baseline pooled 7x30 beat windows

^ corrected percentile method of bootstrap, otherwise bias corrected and accelerated percentile method

p1 < 0.006 baseline compared to A.Resting

p2 < 0.006 (or ‘ p2 < 0.05) for difference between O_2 mask baseline and a: F1, b: F2, c: F3, d: F4, e: F5, f: F6, g: F7, h: F8, i: F9 and j: F10.

a) Meditation and O_2 mask similar, sleep different

b) Sleep and O_2 mask similar, meditation different

c) O_2 mask different to sleep and meditation

d) All different from each other

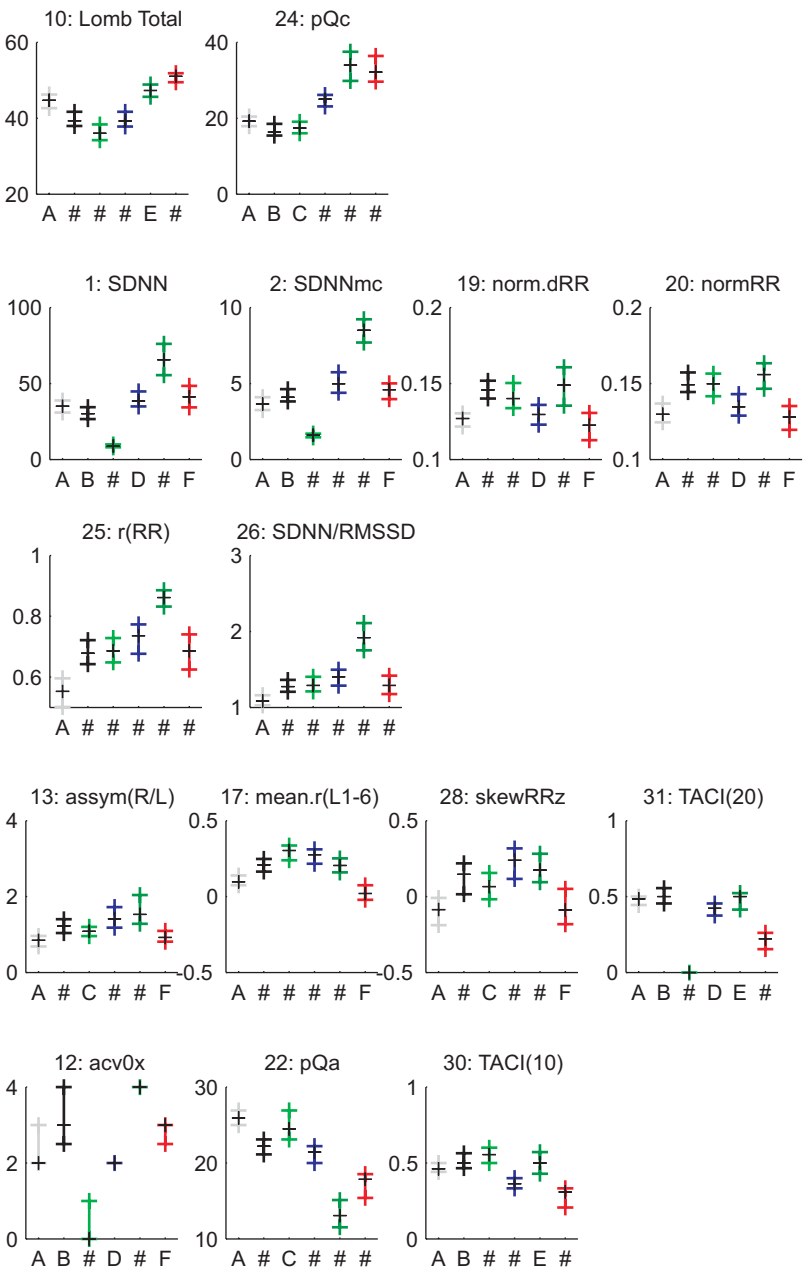


Figure 8-9 O_2 mask (F) indices compared to other physiological states (A-E) , bootstrapped 95% confidence intervals (10x30 beat windows) of the median for selected indices. Significant differences indicated by # p<0.006 replacing database identifier for comparison to A. Resting, B active, C exercise, D sleep, and E meditation.

8.5 Effect of fentanyl dose on extent of respiratory depression

A plot of the fentanyl and midazolam doses with the respiratory depression placed in order of clinically observed depth and length shows the extent of respiratory depression by clinical observation could not be predicted by the dose (Figure 8-10).

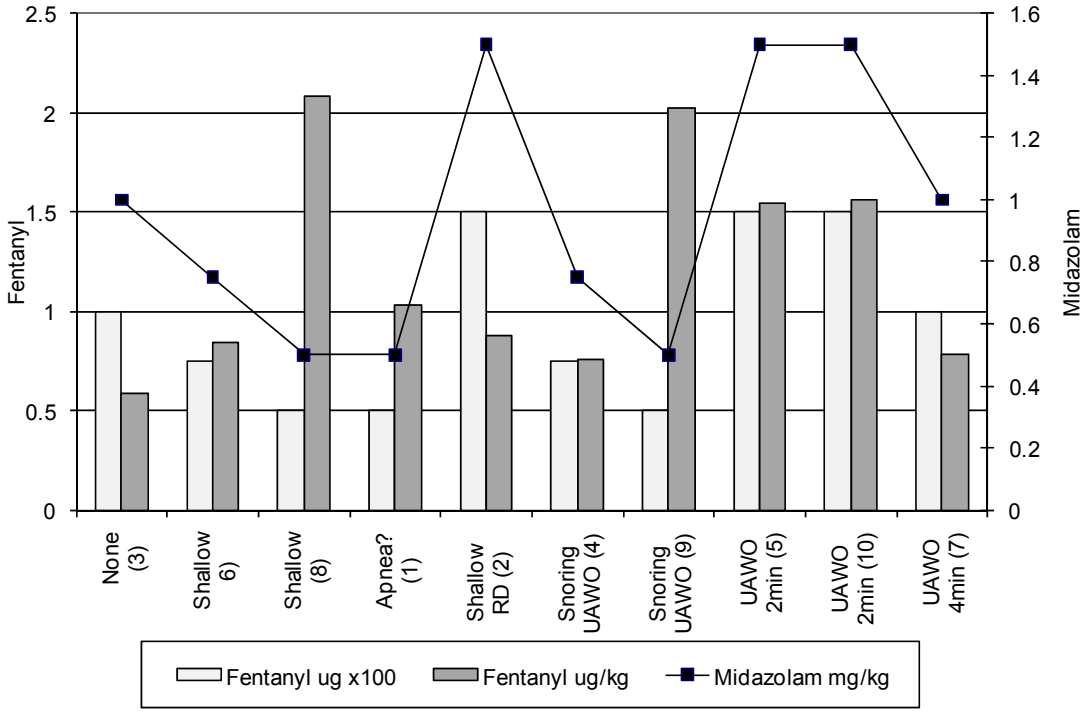


Figure 8-10 Effect of fentanyl and midazolam dose on extent of respiratory depression, from left to right: no clinically observed respiratory depression to sustained full UAWO (subject number in parentheses). Abbreviation: RD, respiratory depression.

8.6 Effect of fentanyl on HRV

The effect on HRV was minimal in the 300 beats after fentanyl administration with four indices, **TACI(10)**, **mean.r(L1-6)**, **LombTotal**, and **normRR** reaching statistical significance (Table 8-7) in the 4th, 5th, 6th and 7th periods after fentanyl administration respectively.

For the 310 post-fentanyl tests, only these four indices with five results remained after correction for multiple tests (Figure 8-11).

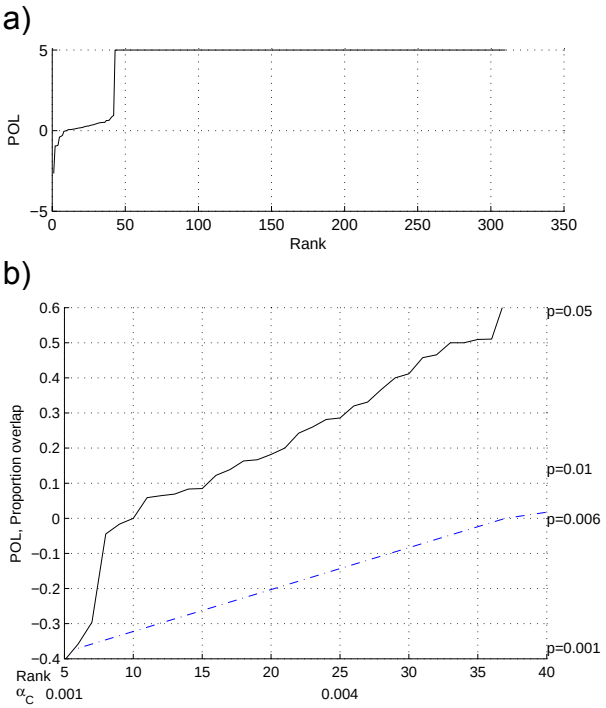


*Figure 8-11 Ranked POL for 310 post-fentanyl tests : a) results for 310 tests maximum POL limited to 5, and b) zoom to FDR cut-off. Corrected alpha, α_C = alpha/n*rank. Right hand axis gives equivalent p-values for key confidence interval overlaps.*

The nonsignificant results were retained to provide context for the significant results. For two of the indices, **LombTotal** and **mean.r(L1-6)**, the significant result was part of a general trend that can be seen in plots of the median (Figure 8-12). The isolated results for **normRR** and **TACI(10)** may indicate a spike or a slight baseline shift only reaching occasional significance.

There was no evidence of changes to the standard indices but there was a median trend for consecutive post-fentanyl periods to decreased total variability (**SDNN**, **LombTotal**) and vagal activity (**RMSSD**, **pNN20**) occurring 60 beats after fentanyl administration.

Although not reaching statistical significance, one other index showed a consistent difference from baseline for multiple, consecutive, post-fentanyl periods: **PolVar20**, a nonlinear index identified short phases of low variability (less than 20 ms between successive RR-intervals) with a sharp rise followed by a fall 180 beats after fentanyl administration.

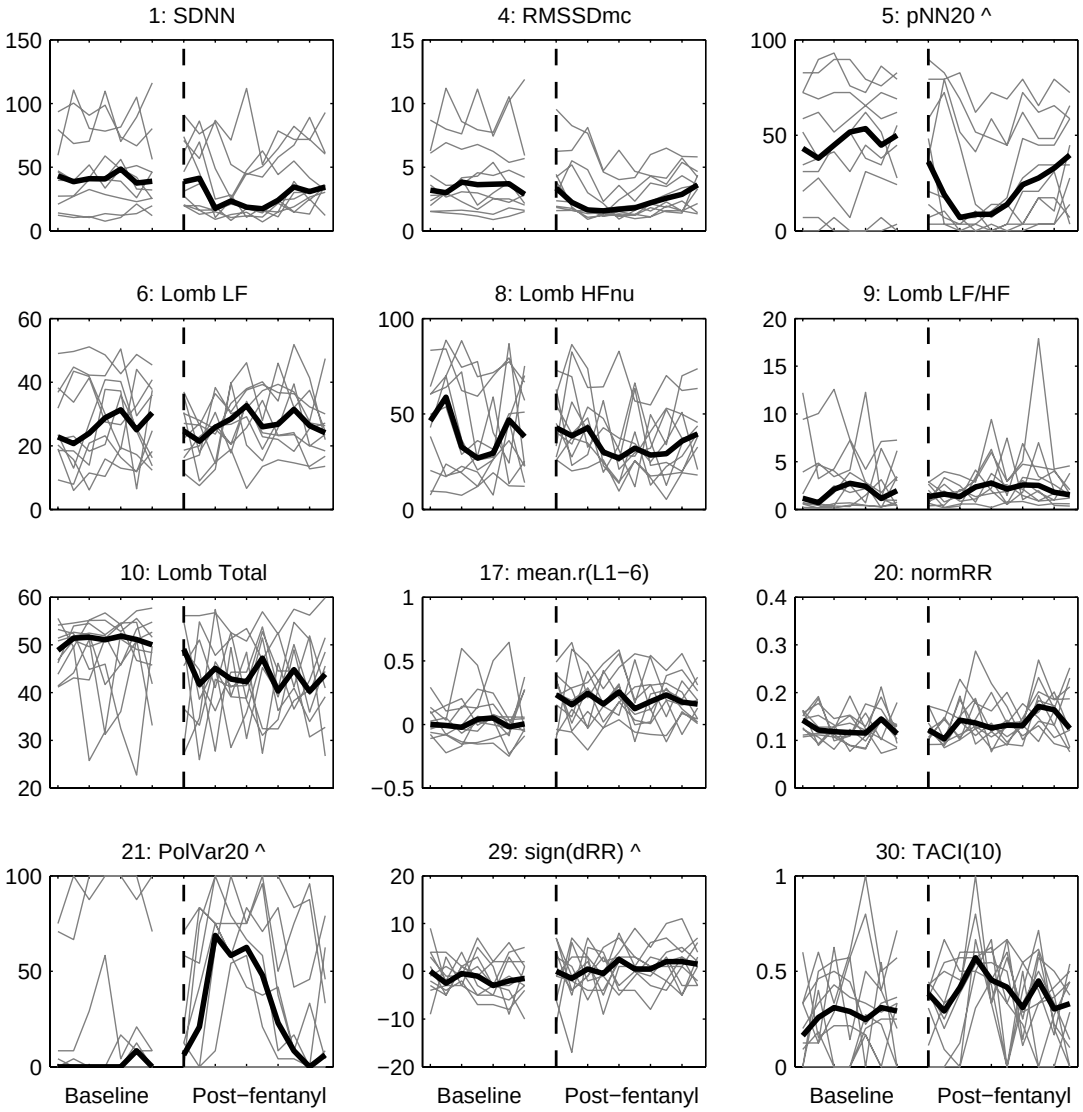


Figure 8-12 HRV response to fentanyl administration, median (thick line) for 10 subjects (thin lines) over 30 beat windows (x-axis: baseline 210 beats, post-fentanyl 300 beats).

8.7 Peri-respiratory depression

8.7.1 Peri-CD

The initial respiratory depression event seen in all subjects was CD. Preceding the onset of CD, three indices had seven significant results (confidence intervals in Table 8-8) after correction for multiple tests (Figure 8-15 a). These three indices, **LombTotal**, **pQa** and **TACI(10)**, show a slight shift in baseline levels that reach occasional statistical significance (Figure 8-13).

Sustained (but statistically nonsignificant) changes were seen again in both **pNN20** and **PolVar20** starting 90 beats before CD. Another index, **TACI(20)**, had a biphasic spike 30 beats before CD.

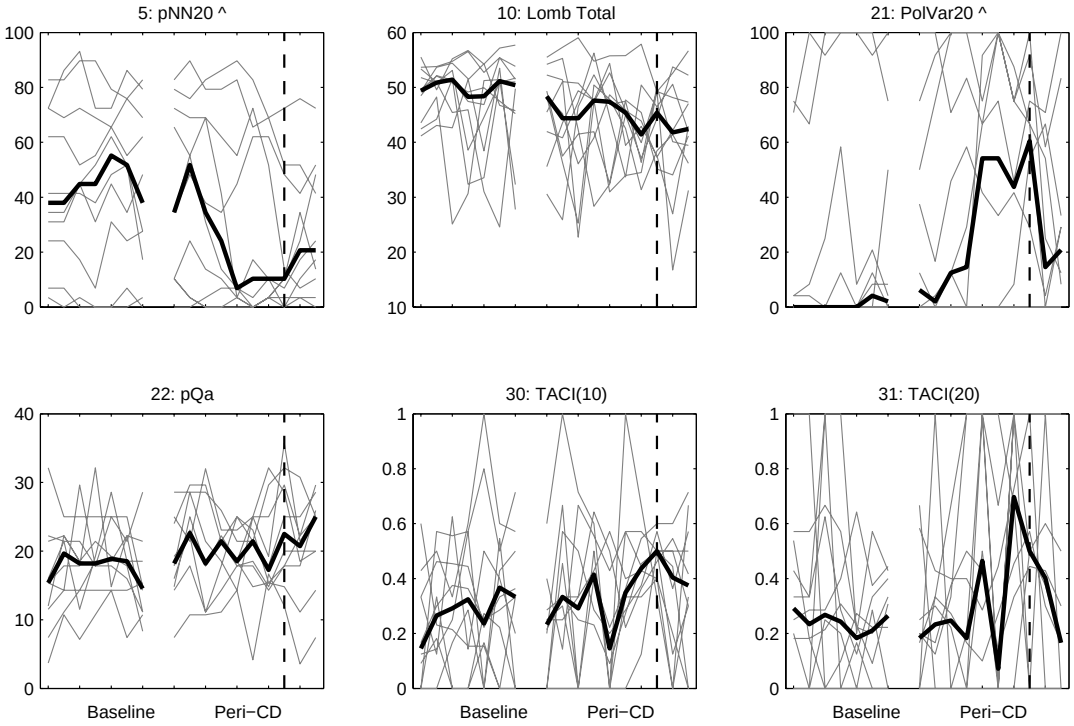


Figure 8-13 HRV response for peri-CD, median (thick line) for 10 subjects (thin lines) over 30 beat windows (x-axis: baseline 210 beats, peri-CD 300 beats). Initial CD at dashed line. Note: The period before CD variably overlapped the baseline for all subjects

Table 8-8 Peri-CD 95% confidence intervals of indices for baseline and 10 consecutive 30-beat intervals aligned at CD (every third interval shown)

Index	Baseline[a]	CD-4 (d)	CD-2 (f)	CD (h)	p
1: SDNN	34.8–49.7	14.5–61.7	13.3–68.6	19.5–45.0	
2: SDNNmc	4.00–5.08	2.10–5.86	1.88–6.29	2.14–4.94	
3: RMSSD	22.6–39.3	11.0–45.4	8.71–55.35	9.41–36.79	
4: RMSSDmc	2.74–4.22	1.62–4.42	1.22–5.07	1.33–3.71	
5: pNN20 ^	32.8–53.4	5.17–62.07	0.00–65.52	1.72–48.28	i'
6: Lomb LF	21.0–32.0	15.4–36.2	14.4–32.0	24.1–32.9	
7: LombHF	12.2–23.1	11.5–29.4	9.68–34.02	7.80–23.63	i'
8: Lomb HFnu	26.0–50.1	26.0–62.2	23.5–64.9	20.9–48.3	
9: Lomb LF/HF	1.00–2.84	0.61–3.12	0.54–3.25	1.08–3.78	
10: LombTotal	48.8–51.4	41.9–52.3	39.4–47.6	37.6–49.3	c',f,g,h',i',j
11: accel	0.48–0.55	0.39–0.58	0.47–0.57	0.53–0.70	h'
12: acv0x	2.00–3.00	1.00–4.00	2.00–6.00	1.50–5.50	
13: assym(R/L)	0.73–1.07	0.57–0.95	0.62–1.67	1.11–4.02	a',h
14: CVdRR	125–129	117–131	118–126	119–147	g',j'
15: gradRR	-0.45–0.38	-0.07–2.85	-3.84–1.55	0.34–2.38	d',h'
16: kurtRRz	2.18–2.56	1.91–2.81	2.38–3.01	2.08–4.49	f'
17: mean.r(L1-6)	-0.01–0.06	0.01–0.25	0.06–0.34	0.01–0.38	f,g,i',j
18: meanRR	757–947	670–1018	676–958	687–1022	
19: norm.dRR	0.11–0.13	0.10–0.15	0.09–0.12	0.10–0.20	
20: normRR	0.11–0.14	0.10–0.16	0.10–0.14	0.10–0.21	c',j
21: PolVar20 ^	0.00–4.17	0.00–83.33	0.00–100.00	0.00–83.33	c',d',e',f', g',h',i',j'
22: pQa	15.1–18.5	18.2–22.6	16.3–25.0	16.3–29.6	b,d',j
23: pQb	25.0–30.9	25.4–39.3	25.9–35.7	14.8–32.1	
24: pQc	29.6–35.2	17.9–34.5	17.9–32.1	21.4–32.7	i',j'
25: r(RR)	0.61–0.74	0.51–0.80	0.55–0.80	0.47–0.87	i'
26: SDNN/RMSSD	1.16–1.42	1.09–1.63	1.07–1.63	1.04–2.04	i'
27: skew.dRR	0.68–0.92	0.27–1.51	0.17–1.08	0.52–1.76	j'
28: skewRRz	-0.21–0.08	-0.38–0.27	-0.22–0.50	-0.21–1.11	
29: sign(dRR) ^	-2.50–0.00	-0.50–7.00	-1.00–5.00	-3.50–1.50	d',f'
30: TACI(10)	0.21–0.33	0.20–0.56	0.27–0.57	0.46–0.59	g,h
31: TACI(20)	0.18–0.29	0.00–0.60	0.00–0.44	0.00–1.00	g'

Note: Correction for multiple comparisons removed (greyed) all but seven significant results for three indices

[a] baseline pooled 7x30 beat windows

^ corrected percentile method of bootstrap, otherwise bias corrected and accelerated percentile method

p<0.006 (or ‘ p<0.05) for difference between baseline and peri-CD windows: a, CD-7; b, CD-6; c, CD-5; d, CD-4; e, CD-3; f, CD-2; g, CD-1; h, CD; i, CD+1; j, CD+2.

8.7.2 Peri-UAWO

All subjects had a critical respiratory event for the second analysis, peri-UAWO. Preceding the UAWO event, four indices showed a difference from the baseline (Table 8-9) after correction for multiple tests (Figure 8-15 b):

- One statistically significant event in 10 peri-RD periods: **accel.**
- Significant event was one of multiple near significant events: **mean.r(L1-6), TACI(10), TACI(20)**.

Sustained (but statistically nonsignificant) trends starting 120 beats before UAWO were seen in **pNN20**, and **PolVar20** (Figure 8-14).

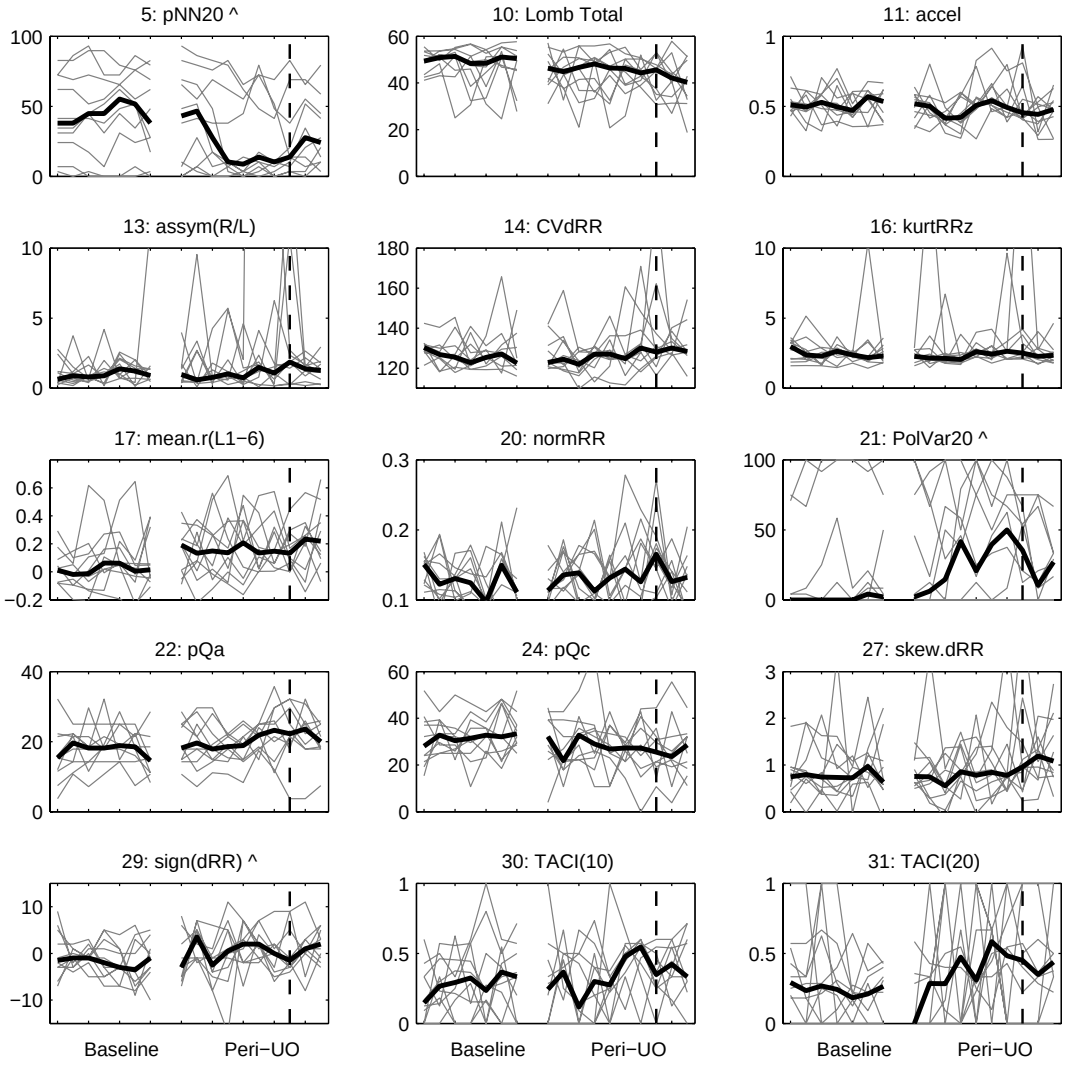


Figure 8-14 HRV response for peri-UAWO median (thick line) for 10 subjects (thin lines) over 30 beat windows (x-axis: baseline 210 beats, peri-UAWO 300 beats). Note: The period before UAWO (dashed line) variably overlapped the baseline for all subjects.

Table 8-9 Peri-UAWO 95% confidence intervals of indices for baseline and 10 consecutive 30-beat intervals aligned with 8th period at UAWO (every third interval shown)

Index	Baseline[a]	UO-4 (d)	UO-2 (f)	UO (h)	p
1: SDNN	34.8–49.7	18.5–61.9	17.4–70.3	15.8–49.0	
2: SDNNmc	4.00–5.11	2.59–7.38	2.20–7.21	2.05–5.58	g'
3: RMSSD	22.6–39.6	11.0–45.5	10.7–51.1	12.4–37.8	
4: RMSSDmc	2.72–4.20	1.68–4.84	1.40–5.00	1.60–3.62	
5: pNN20 ^	32.8–55.2	5.17–62.07	5.17–72.41	5.17–37.93	h'
6: Lomb LF	21.0–31.4	18.6–34.2	13.4–43.7	14.7–37.6	
7: LombHF	12.3–24.0	10.2–32.4	6.44–24.60	8.36–27.46	i'
8: Lomb HFnu	26.0–49.6	22.8–63.6	12.8–55.0	15.7–61.2	
9: Lomb LF/HF	0.95–2.84	0.57–3.61	0.71–6.96	0.56–4.50	
10: LombTotal	48.8–51.4	36.7–50.5	39.0–51.3	35.1–50.3	g',h',i,j'
11: accel	0.48–0.55	0.33–0.58	0.49–0.64	0.41–0.65	c
12: acv0x	2.00–3.00	0.00–4.00	0.00–4.00	1.00–4.00	
13: assym(R/L)	0.73–1.07	0.68–3.37	0.52–2.90	0.32–6.31	
14: CVdRR	125–129	120–130	120–130	123–147	i'
15: gradRR	-0.45–0.38	-3.08–1.63	0.28–3.63	-1.30–3.28	f'
16: kurtRRz	2.18–2.56	1.89–2.38	2.11–2.97	2.24–3.80	
17: mean.r(L1-6)	-0.01–0.06	-0.00–0.36	0.01–0.32	0.04–0.27	g',h',j
18: meanRR	757–947	671–1018	714–1021	731–1033	
19: norm.dRR	0.11–0.13	0.09–0.16	0.09–0.15	0.10–0.20	
20: normRR	0.11–0.14	0.10–0.17	0.10–0.18	0.13–0.21	h'
21: PolVar20 ^	0.00–2.08	0.00–64.58	0.00–66.67	0.00–60.42	b',c',d',e',f',g',h',i',j'
22: pQa	15.4–18.5	14.8–21.4	17.9–25.0	18.5–29.6	f',h,i',j'
23: pQb	25.0–30.9	23.5–42.3	25.0–38.2	14.8–39.3	b'
24: pQc	29.6–34.6	21.4–35.7	21.7–29.1	16.8–32.1	f
25: r(RR)	0.61–0.74	0.58–0.82	0.64–0.84	0.43–0.84	i'
26: SDNN/RMSSD	1.15–1.42	1.11–1.87	1.22–1.79	1.01–1.74	i'
27: skew.dRR	0.68–0.92	0.71–1.44	0.34–1.32	0.47–1.78	
28: skewRRz	-0.21–0.08	-0.33–0.37	-0.42–0.41	-0.66–0.94	
29: sign(dRR) ^	-2.00–1.00	-3.50–6.00	0.00–7.00	-3.00–7.00	f'
30: TACI(10)	0.21–0.33	0.00–0.50	0.36–0.56	0.25–0.50	f,g
31: TACI(20)	0.18–0.29	0.00–1.00	0.33–0.67	0.00–1.00	a,f,j

Note: Correction for multiple comparisons removed (greyed) all but five results for four indices. Abbreviation: UO, upper airway obstruction

[a] baseline pooled 7x30 beat windows

p<0.006 (or ‘ p<0.05) for difference between baseline and peri-UAWO periods: a, UO-7; b, UO-6; c, UO-5; d, UO-4; e, UO-3; f, UO-2; g, UO-1; h, UO; i, UO+1; j, UO+2.

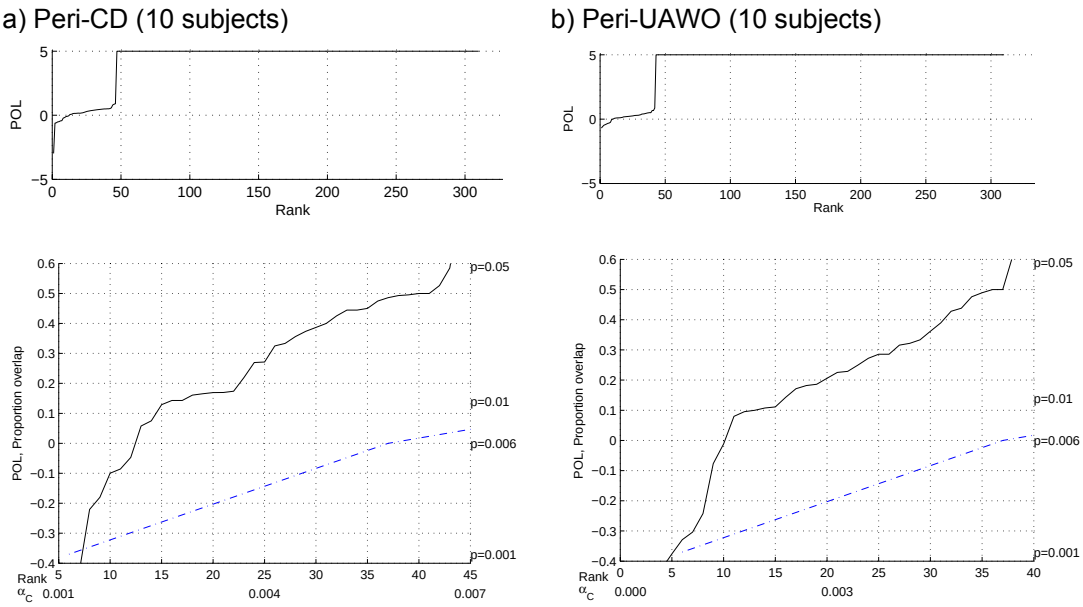


*Figure 8-15 Ranked POL a) peri-CD and b) peri-UAWO: (top) results for 310 tests, maximum POL limited to 5, (bottom) zoom to FDR cut-off. Corrected alpha, α_C = alpha/n*rank. Right hand axis gives equivalent p-values for key confidence interval overlaps*

8.8 Individual differences in response to respiratory depression events

The individual subject responses for a selection of indices were visually inspected for differences. As well as the traditional time domain and spectral indices, significant and interesting indices were inspected in three groups:

- Significant with no spikes: **accel, pQa**, **TACI(10)**, and **TACI(20)**.
- Spikes peri-RD: **assym(R/L)**, **CVdRR**, **mean.r(L1-6)**, and **normRR**.
- Sustained changes: **pNN20**, and **polVar20.**

The plots are grouped according to the extent of UAWO: the four subjects who had RRspiro = 0 but no events with $ETCO_2$ = 0 (left 4 plots); the three subjects with UAWO ($ETCO_2$ = 0) that was not clinically observed (3 top right plots); and three subjects with clinically observed UAWO (3 bottom right plots).

In each of the individual response plots, the vertical bars represent: a) mask on; b) start of fentanyl administration; c) initial CD event; d) first occurrence of

no flow, RRspiro = 0 or $ETCO_2$ = 0; and e) clinical observation of snoring or full UAWO.

To provide as much baseline as possible but still retain detail in the post-fentanyl period and keep all plots to the same scale, the x-axis for these plots starts at 460 beats before fentanyl administration and goes to 460 beats after, even when the baseline is not this long.

8.8.1 Time domain indices

The traditional time domain indices **SDNNmc**, **RMSSDmc** and **pNN20**, showed some subjects had large changes, but the changes were not related to the extent of respiratory depression: no flow subjects 4 and 8; $ETCO_2$ = 0 subjects 1 and 9; and clinical UAWO subject 5 (Figure 8-16).

For each index, the three subjects with clinical UAWO (5, 7 and 10) had a different response: decrease, small spike, and small increase respectively.

8.8.2 Spectral indices

Spectral indices, **LombLF**, **LombLF/HF** and **LombTotal**, showed no particular trends for any subjects after fentanyl administration (Figure 8-17). Large drops in **LombTotal** occurred at the time of fentanyl administration in five subjects (1, 2, 6, 7, and 9) with drops about the respiratory depression event in two subjects with clinical UAWO (5 and 7).

Two subjects (6 and 8) with high HFnu baseline levels (after O_2 mask) had a large drop with fentanyl.

Increases in LF power after fentanyl that coincide with decreases in HF power may be caused by the shift in power that occurs with slowing respiratory rate.

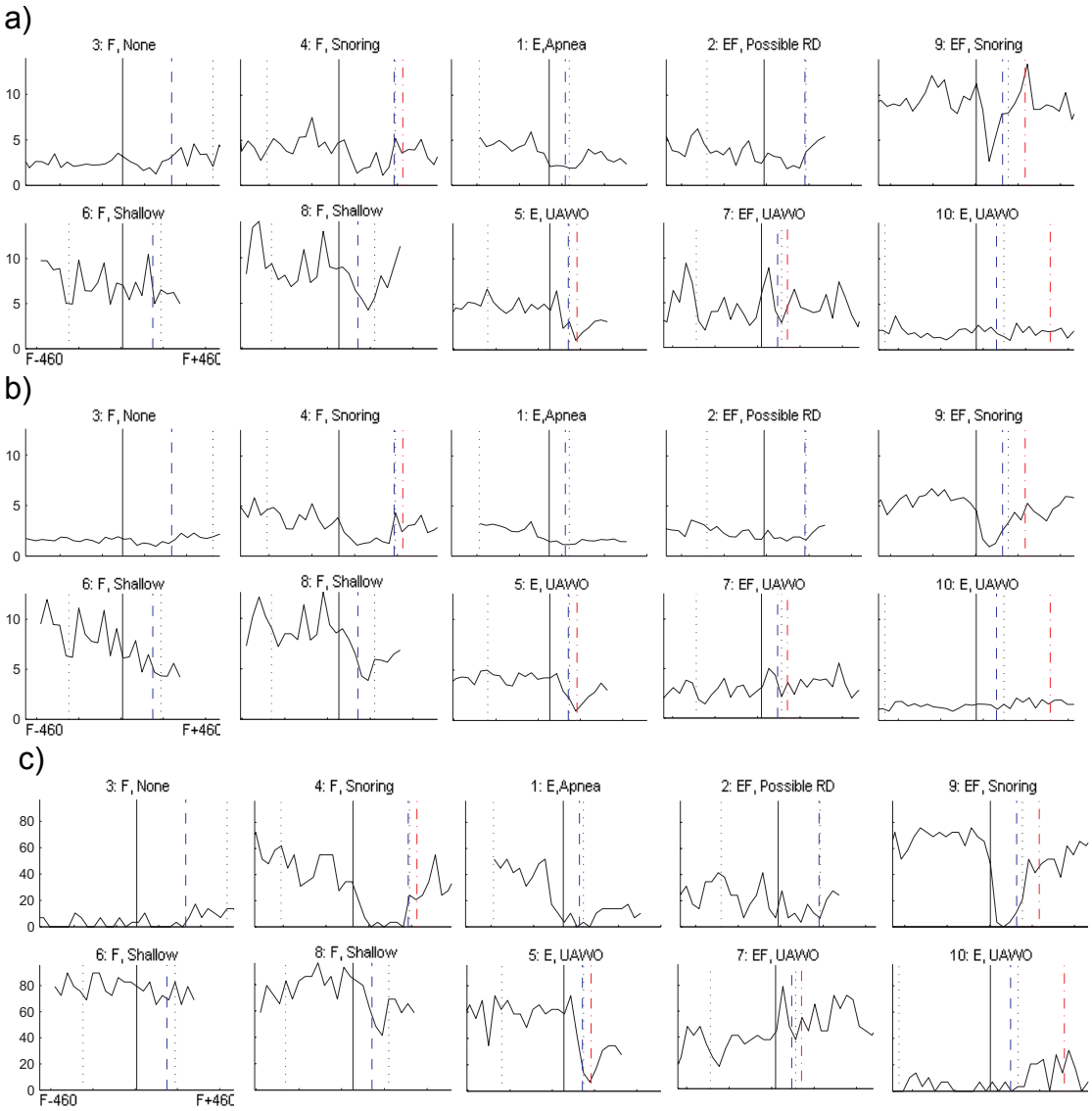


Figure 8-16 Individual responses to fentanyl for time domain indices
a) SDNNmc, b) RMSSDmc and c) pNN20 from 460 beats before (if recorded) to 460 beats after fentanyl.
Vertical lines: mask on (dot); fentanyl (solid); initial CD (dash); UAWO $ETCO_2$=0 or RRspiro=0 (dot), clinical UAWO (dash-dot).
Titles: subject number, UAWO event (F, E, or EF), observed clinical event
Abbreviations: E, $ETCO_2$=0; F, RRspiro = 0.

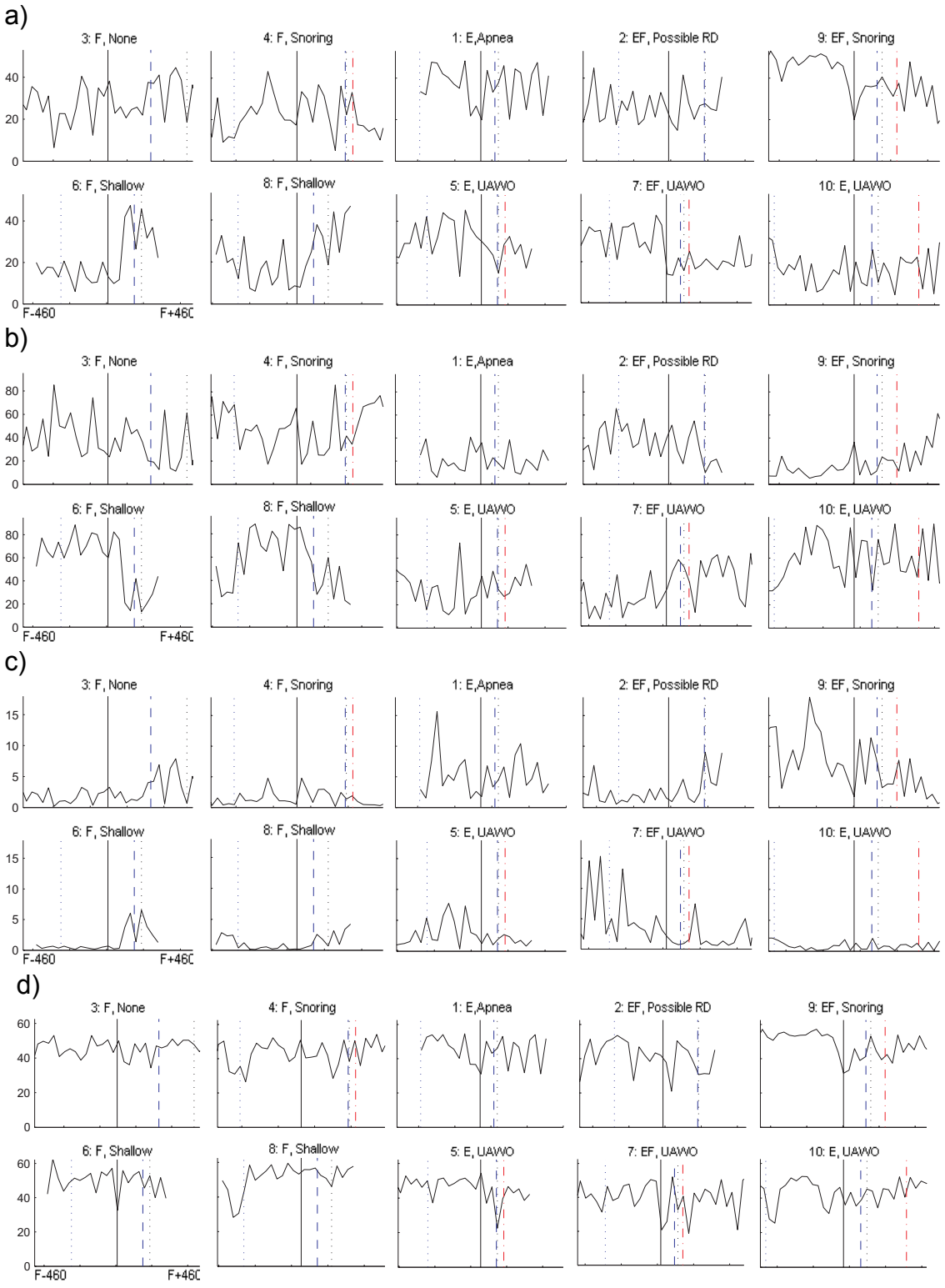


Figure 8-17 Individual responses for Lomb Scargle spectral indices a) LombLF, b) LombHFnu, c) LombLF/HF and d) LombTotal (explanation as for Figure 8-16)

8.8.3 Significant indices

Some significant (in either peri-CD or peri-UAWO analysis) indices had inconsistent spikes around UAWO (Figure 8-18):

- **accel**; accelerations below the Poincaré plot line of identity showed inconsistent changes with no visible pattern around respiratory depression events.
- **pQa**; high scores when decreased RR-interval followed by increased RR-interval, showed a peak for 3 of the 6 UAWO subjects (2, 5, and 7).
- **TACI(10), TACI(20)**; acceleration indices, with thresholds of 10 and 20 ms, also showed inconsistent changes.

8.8.4 Spikes near UAWO

Four indices (only **mean.r(L1-6)** reached statistical significance) had spikes in the period around respiratory events (Figure 8-19):

- **assym(R/L)**; sample asymmetry analysis of the histogram tails, exaggerating transient accelerations and decelerations, showed spikes around the respiratory depression event for some subjects with CD only (4), UAWO (2,9) and clinical UAWO (5), but were largest for those with snoring (4 and 9).
- **CVdRR**; the coefficient of variation which minimises the effect of heart rate on variability also showed spikes around the respiratory depression event for some subjects with CD only (4), UAWO (9), and clinical UAWO (5,7).
- **mean.r(L1-6)**; the mean of correlation coefficients, r, for lagged plots from one to six lags showed inconsistent changes except for snoring.
- **normRR**; normality of the RR-intervals showed large peaks for the snorers (4 and 9) and subject 2.

The occurrence of these spikes was spread over the different respiratory depression events rather than occurring more with greater depth of respiratory depression. The spikes were largest preceding a snoring event. Spikes for subjects 2, 4, and 6 occur on, or close to the defined CD and UAWO events

even though there is the possibility (due to missing data) the event may have occurred before this time.

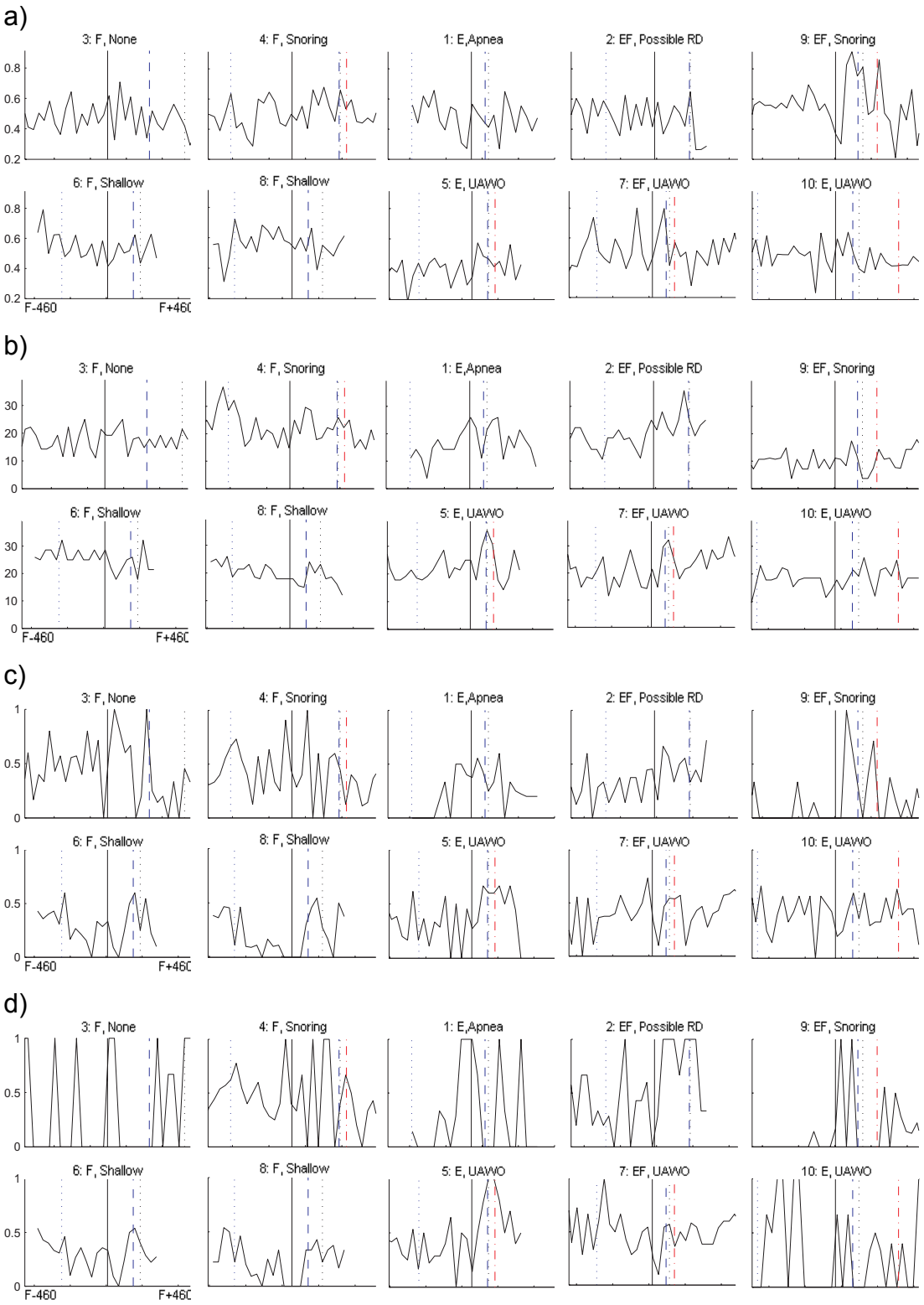


Figure 8-18 Individual responses for significant indices a) accel, b) p.Qa, c) TACI(10), and d) TACI(20) (explanation as for Figure 8-16)

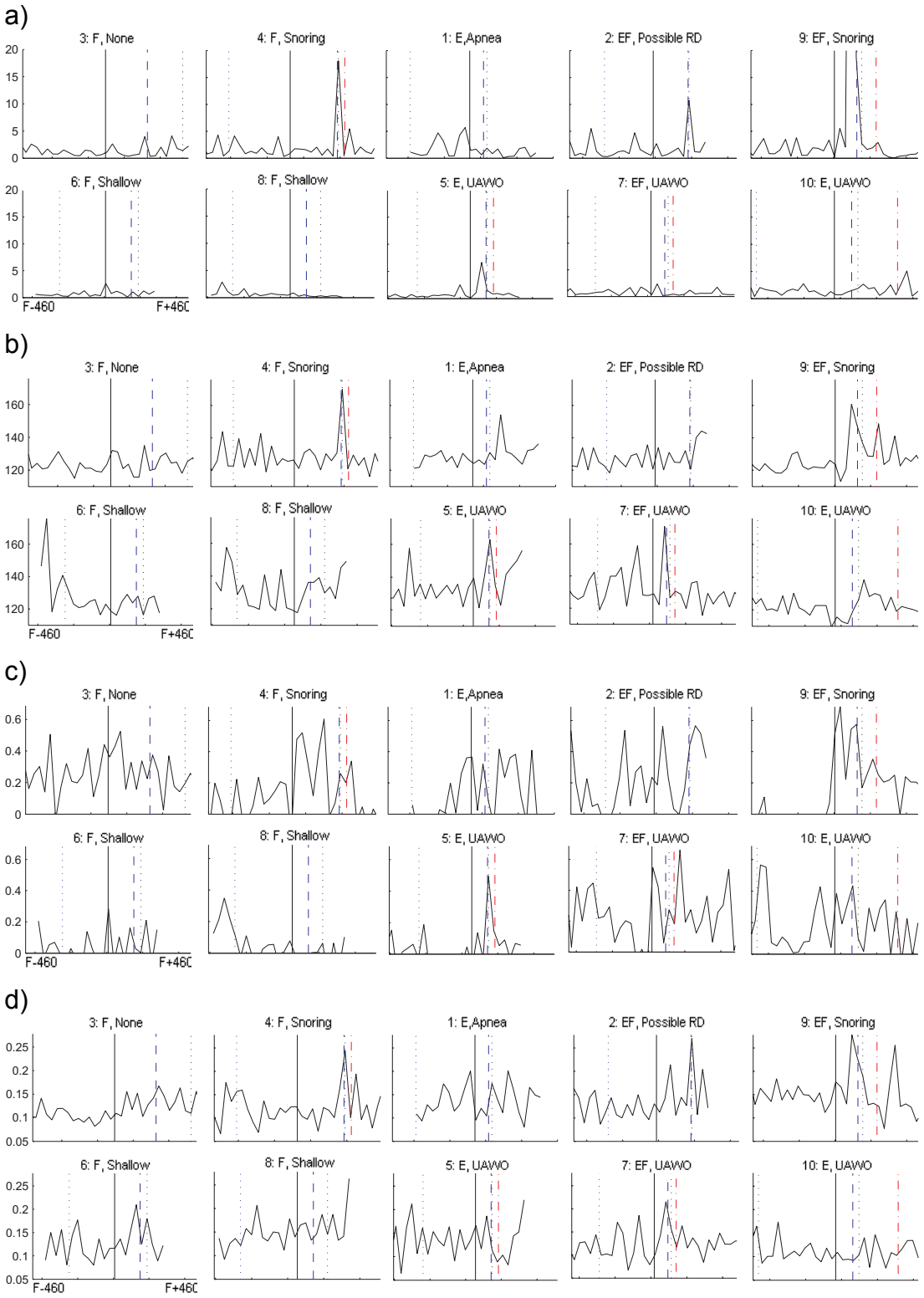


Figure 8-19 Individual responses for indices with spikes
a) assym(R/L), b) CVdRR c) mean.r(L1-6), and d) normRR
(explanation as for Figure 8-16)

8.8.5 Sustained peaks or dips

Sustained changes were seen in some indices which reached statistical significance only occasionally: **pNN20** (Figure 8-16 c) and **PolVar20** (Figure 8-20).

pNN20

This index identifies periods of high variability, producing a smaller scale of measurement as variability decreases. Baseline HRV characteristics have an effect on **pNN20**: it will not register for subjects with normally high variability, which is why the family of pNNxx has been developed over the years.

PolVar20

PolVar20 identifies short phases of low variability, with less than 20 ms between successive RR-intervals. A large sustained increase with fentanyl administration occurred in half the subjects (1, 4, 5 and 9) and these all had very low baseline levels of **PolVar20**. A decrease occurred with the onset of respiratory depression in seven of ten subjects (1, 2, 3, 4, 5, 9 and 10) regardless of the extent of respiratory depression. No **PolVar20** response was seen for subjects 6, 7 and 8.

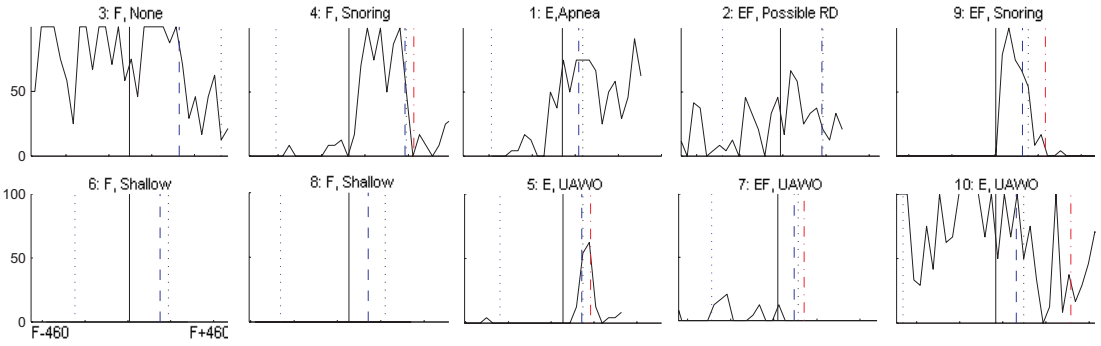


Figure 8-20 Individual responses to fentanyl for PolVar20 (explanation as for Figure 8-16)

The **PolVar20** response was not related to the type of obstruction: the three subjects that had sustained full obstruction (5, 7 and 10) had different individual **PolVar20** responses with no response from subject 7.

Some of the different responses of **PolVar20** can be linked to the baseline HRV. Subjects with no baseline **PolVar20** (6, 8) had higher baseline **SDNN**, and those with high baseline **PolVar20** had low **SDNN** (Table 8-10).

Table 8-10 Individual subject HRV parameters affecting PolVar20

Subject		Baseline		PolVar20
	meanRR	SDNN	RMSSD	
1	893	41.2	26.0	
2	709	27.4	17.5	Baseline moderate
3	630	14.2	10.3	Baseline high
4	731	35.0	27.0	
5	1015	50.7	44.0	Baseline tiny
6	1174	88.9	97.9	Not registered
7	998	35.8	26.2	
8	978	84.1	87.8	Not registered
9	821	83.2	49.3	Not registered
10	708	12.7	9.7	Baseline high
Mean	866 (174)	47 (29)	40 (31)	

Abbreviation: meanRR, mean RR-interval.

The plot of these parameters shows that the biggest effect is from **SDNN** (Figure 8-21). Changing the threshold from 20 ms improved the ability of this low variability index to identify change.

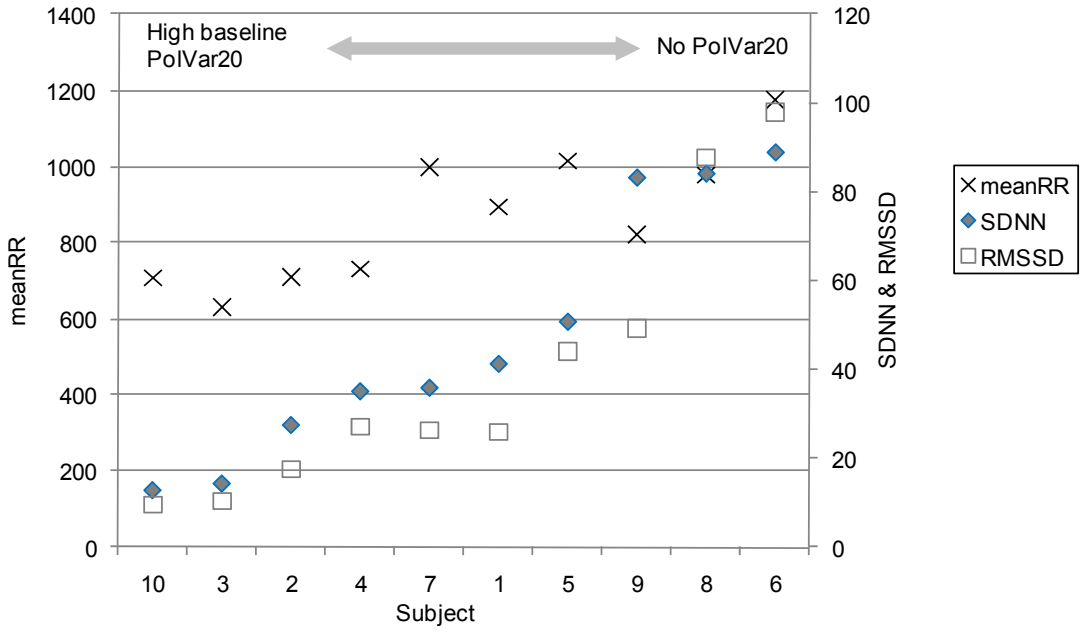


Figure 8-21 Plot of subject RR-interval parameters affecting baseline PolVar20 (ordered by SDNN)

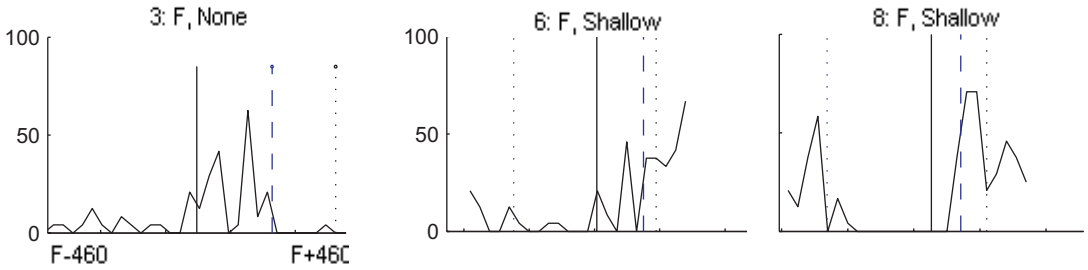


Figure 8-22 PolVarXX for subjects with high and low baseline SDNN : PolVar10 (subject 3), and PolVar80 (subjects 6 and 8)

For subject 3 with a high baseline **PolVar20**, changing to a 10 ms threshold, **PolVar10**, enabled baseline changes to be observed. For subjects 6 and 8 with no response, using an 80 ms threshold, **PolVar80**, showed a change with respiratory depression (Figure 8-22).

For subject 8, the baseline spike occurred with mask application at 178 beats. The drop towards the end of the record coincided with a pager alarm that may have disrupted the descent of respiratory depression and prevented the subject from reaching an $ETCO_2$ of 0, or clinical UAWO.

PolVar20 had a sustained peak yet only reached statistical significance occasionally. This is a result of the distribution: a reverse J, or ski slope distribution, with large numbers of zeros (i.e. the lower confidence limit and the median were both zero). With this distribution, the confidence intervals for the consecutive time periods always overlapped and thus reduced the statistical significance obtainable. This effect is also true of other indices with a skewed distribution that result in many zeros and with a sustained response that did not reach statistical significance (e.g. **pNN20)**.

8.8.6 Consistency of individual responses

A summary of the visually inspected individual responses for each index (Table 8-11) showed that the heart rate variability of some subjects (4, 5, and 9) was consistently more responsive than others, even if only CD and no UAWO event occurred. Two of these were snorers (4 and 9).

Table 8-11 Summary of individual responses to selected indices with large changes about the respiratory depression event that began after fentanyl administration

	No UAWO				UAWO			Clinical UAWO		
Index	3	4	6	8	1	2	9	5	7	10
SDNNmc		Y		Y	Y		Y	Y		
RMSSDmc		Y		Y			Y	Y		
pNN20		Y		Y	Y		Y	Y		
LombLF			Y	bY				bY	Y	
LombHFnu			Y	Y						
LombLF/HF	Y		Y			bY				
LombTotal								Y	Y	
accel						Y	Y	Y		
assym(R/L)		Y				Y	Y	Y		rY
CVdRR		Y			rY	Y	Y	Y	Y	Y
mean.r(L1-6)		Y					Y	Y	Y	
normRR		Y	bY			Y	Y	rY	Y	
pQa						Y		Y	bY	
TINN10, 20	Y	Y					Y	rY		
PolVar20/80	Y	Y		Y	Y		Y	Y		Y

Abbreviations: bY, large response but within baseline range; rY, response after respiratory events; Y, large response.

8.9 Student t-test

The paired Student's t-test was not performed. The large range of event times meant there was insufficient baseline time to allow for the sham F-baseline to precede fentanyl administration by the same amount the RD-baseline had to precede the respiratory depression event and fentanyl administration.

Ignoring the subject with the longest maximum time from the start of fentanyl administration to a respiratory depression event as an outlier, the next longest was 259 beats (for CD). Adding 30 beats to avoid the activity preceding drug

administration set the RD-baseline end point at 259 beats before the respiratory depression event. Subsequently, the F-baseline (sham) also needed to finish 259 beats before fentanyl administration. Even with a short 30-beat window for the baseline (not optimal, longer window preferred) only half the subjects had enough baseline time to fit this in.

8.10 Results summary

Ten sets of data (8 female) were analysed with an average fentanyl dose of 1.2 $\mu g \cdot kg^{-1}$ (SD 0.55) and midazolam 0.032 $mg \cdot kg^{-1}$ (SD 0.005). ECG quality was good with no ectopic or other unusual beats affecting the RR-intervals.

Clinical observation confirmed snoring in two subjects and sustained full UAWO in three subjects. The remaining subjects had lesser respiratory depression events: machine apnoea (1), unconfirmed (1), shallow respirations (2), and no observed effect (1).

There was no evidence of hypoxia or hypercapnia: SpO_2 remained above 96% throughout the study once the O_2 mask was in place (for the eight of ten subjects with data); and $ETCO_2$ > 45 mmHg occurred only toward the end of the study after the peri-RD analyses.

Subsequent analysis of physiological data showed an initial CD event in all subjects. All subjects went on to have UAWO or a critical respiratory depression event where RRimped could not confirm UAWO. There were difficulties with the analysis of the physiological data: baseline $ETCO_2$ not normal, occasional mask leaks, and missing data collection. These affected the identification of the start of the respiratory depression events and the confirmation of UAWO.

From the start of fentanyl administration, CD took on average 138 beats and UAWO 180 beats with clinical observation of any respiratory depression event taking 213 beats. The minimum times were 76, 89 and 123 beats respectively.

The oxygen mask had an effect on HRV compared to supine rest: decreased **meanRR**, and increased total variability **LombTotal** and sympathovagal

balance **SDNN/RMSSD**. These baseline effects may hide or inflate the development of fentanyl effects.

The dose of fentanyl or midazolam had no apparent effect on the extent of the clinically observed respiratory depression.

In the 300 beats after fentanyl administration, the effect on HRV was minimal with four indices showing indiscriminate responses with slight baseline shifts: **LombTotal**, **mean.r(L1-6)**, **normRR**, and **TACI(10)**.

There was no evidence of changes to spectral power but there was a median trend (Figure 8-12) to decreased total variability (**SDNN**, **LombTotal**) and vagal activity (**RMSSD**, **pNN20**) 60 beats after fentanyl administration.

Preceding the onset of the initial CD, three indices had a significant response: slight baseline shifts in **LombTotal**, **pQa** and **TACI(10)**. Of interest, but not reaching statistical significance, were **PolVar20**, with a sustained peak preceding CD by 90 beats, and **pNN20** that also dropped at this time.

For the peri-UAWO analysis, four indices showed a difference from baseline: one had a single significant event: **accel**; the others had a significant event that was one of multiple near-significant events: **mean.r(L1-6)**, **TACI(10)**, and **TACI(20)**. Sustained trends starting 120 beats before UAWO were seen in **LombTotal**, **PolVar20** and **pNN20**.

Investigation of the individual subject responses with the traditional indices: **SDNNmc**, **RMSSDmc** and **pNN20**, showed some subjects (five of ten) had large changes for all three indices, but the changes were not related to the extent of respiratory depression. Of the three subjects with clinical UAWO, they had a decrease, a small spike, and a small increase respectively. The spectral indices had no specific trends for any subjects.

For the indices with spikes the occurrences were inconsistently spread over the different respiratory depression events rather than occurring more in subjects with deeper respiratory depression. The spikes were largest for the

two snorers and missing data for some subjects did not affect the spike location relative to respiratory events.

A summary of the individual responses for each index showed that the heart rate variability of some subjects was consistently more responsive than others, even if only CD and no UAWO event occurred.

One index, although only reaching occasional statistical significance, consistently showed a difference from baseline for multiple, consecutive, post-fentanyl periods: **PolVar20** identified short phases of low variability (less than 20 ms between successive RR-intervals) with a sharp rise followed by a fall 180 beats after fentanyl administration. A drop for this period was also recorded by **pNN20**, but was less clearly defined than **PolVar20**.

Further investigation of **PolVar20** showed two issues with its use: its response depended on the baseline **SDNN** and the highly skewed distribution with large numbers of zeros (lower confidence interval and median both zero) reduced the statistical significance obtainable using the proportion overlap of confidence intervals. These are also issues for the other indices such as **pNN20**.

Chapter 9. Discussion for HRV of fentanyl-induced loss of airway tone

9.1 Study design

The hypothesis was that a measure of short-term, non-stationary HRV could detect a change in vagal or sympathetic activity that may be a reflection of a shift in the stability of airway tone. The original problem identified opioid-induced loss of airway tone as a cause of critical respiratory depression.

The preferred use of fentanyl in the pre-operative setting necessitated measuring HRV over short periods (<60 s). Although the common use of fentanyl in this situation simplified logistics (making use of subjects already scheduled for fentanyl administration) and ensured subject safety with the presence of the anaesthetist throughout, its fast time to peak effect altered the measurement of HRV requiring indices capable of measurement over periods much shorter than that normally used, with the period decreased from the widely used 5 min down to 30 beats.

HRV indices had to be selected, tested and validated over the short 30-beat windows with physiological data to prove their effectiveness at discriminating physiological states and remove redundant indices, before being applied in this study.

9.2 Fentanyl effect on HRV

It is generally accepted by clinicians that opioids reduce sympathetic and enhance vagal (decreased heart rate) and parasympathetic tone (decreased respiratory rate and depth, and depressed upper airway reflexes) with a mixture of effects on different organs [6] and the expectation in this study was to look for increased vagal and parasympathetic activity.

Published HRV studies of fentanyl effects (summary in Chapter 3.6.3) on the cardiovascular system have shown it decreased total, and LF power, sometimes heart rate and conversely often decreased HF power [157, 243, 247, 251-254], with few studies showing increased relative vagal tone by an

increased HFnu [250, 252, 254]. The effects on HF, HFnu and LF/HF power are variable. The same results (though less consistent) are found when any opioid is part of an anaesthetic package with decreased LF and total spectral power [160, 232-234, 236, 239]. These studies reported on spectral HRV indices over periods of 5 to 15 min.

Use of very short-term 30-beat assessment in this study did not find the expected fentanyl-induced decreases in heart rate, total variability, or sympathetic tone by the indices commonly used for this purpose over longer periods, nor did it find any change in vagal tone (absolute or relative).

Possible reasons for this include: 1) fentanyl had not reached peak effect; 2) known fentanyl effects are contradictory; 3) low-dose midazolam; 4) concomitant effect of midazolam with opioids; 5) anxious subjects with high sympathetic activity; 6) baseline vagal activation of oxygen mask; 7) respiratory depression effects; 8) inability of 30-beat indices to detect effect; and 9) pre-existing conditions.

9.2.1 Peak effect of fentanyl

The ability of this study to detect changes caused by fentanyl may have been reduced because the focus of this study was on the development of respiratory depression; it investigated the 5 min immediately following fentanyl administration and may have ended before the peak effect of fentanyl which takes 5-10 min [6], and before loss of consciousness or intubation and mechanical ventilation [251, 252] that most other studies include.

In this study, all subjects had at least one central depression occurring between 1 and 3 min from fentanyl administration. It may be that vagal tone was still changing and had not reached a level that was detectable in this analysis.

9.2.2 Contradictory effects of fentanyl

Contradictory results have been reported investigating fentanyl use with other drugs: some showing vagal predominance (increased HFnu [252, 254] or decreased LF/HF [250, 251]), others showing no change [247, 253] and one

showing an increase in relative sympathetic activity [243], though this may have been caused by positive pressure ventilation after loss of consciousness.

In the only human study on HRV effects of fentanyl in isolation from other drugs, Vettorello *et al.* [247] did not find vagal activation. With a similar low dose (1 μg·kg^{-1}, mean 74 μg) to this study they found sympathetic withdrawal (decreased HR, SDNN, LFnu, LF/HF) and only a trend toward vagal activation (decreasing total power and increasing HFnu) starting 6 min after fentanyl administration with both spontaneous and paced breathing protocols.

This study undertaken in the 6 min preceding where the Vettorello study began shows only some similarities: trend to decreased SDNN, and Lomb Total, but no trends to increased vagal activation (HFnu) or sympathetic withdrawal (LFnu, LF/HF). This again may indicate that the use of an earlier endpoint in this study means vagal and sympathetic activity are yet to reach their full cardiac effect in response to fentanyl.

9.2.3 Low-dose midazolam

This study used low-dose midazolam, mean 0.03 mg·kg^{-1}, much less than the sedative doses (0.1 mg·kg^{-1}) that are known to induce both central depression and upper airway obstruction [6]. While midazolam at higher doses (>0.05 mg·kg^{-1}) has generally been shown to increase heart rate and decrease HRV [448, 467-470] with mixed effects on LF/HF, Schachinger *et al.* [471] showed small doses of midazolam (0.02 mg·kg^{-1}, half by bolus the rest infused over 1 hr to give stable blood plasma levels) increased HRV and increasing doses (0.06 and 0.14 mg·kg^{-1}) decreased HRV with no changes in LF/HF.

In this study, the low-dose midazolam may have increased HRV, counteracting the expected fentanyl effect of decreasing HRV thus reducing the overall response.

9.2.4 Concomitant midazolam and opioids

Midazolam premedication was used in many of the published studies using fentanyl [243, 253] or other opioids [160, 238].

The concomitant administration of benzodiazepines such as midazolam are known to significantly alter both the cardiovascular and respiratory actions of opioids [6]. Latson *et al.* [252] looked for increased vagal activity with high-dose sufentanil[14] (2.9 μg·kg^{-1}, with midazolam premedication 3.5 mg 45 min prior) before intubation and found large decreases in HF as well as total power, LF and LF/HF with loss of consciousness. They did find an increase in HFnu reflecting a change toward vagal balance (though LF/HF still >1).

Riznyk *et al.* [253], 1 hr before induction, used a large dose of midazolam (7.5 mg, mean 0.11 mg·kg^{-1}) and found in the 5 min pre-induction period compared to 5 min post-induction, fentanyl (3 μg·kg^{-1}) decreased sympathetic activity (total power and LF), with no change in HF or the relative powers (HFnu, LFnu and LF/HF). They also started 100% O_2 with facemask 2-3 min pre-induction and made no comment on the effect of this.

The concomitant use of midazolam and fentanyl ensured subjects would reach the end point of respiratory depression with smaller doses of fentanyl at the expense of an undefined overall effect on the HRV.

9.2.5 Anxious subject group

Although the subjects had been supine for 15 min before the study, they were not necessarily relaxed; as could be expected before surgery, they were often anxious and could have enhanced sympathetic activation until fentanyl administration. Studies of HRV investigating anxiety in supine subjects have shown: increased LF/HF [472], increased mean HR, LF, LF/HF with decreased HF [473] and increased heart rate (i.e. decreased meanRR) and SDNN [474].

The effect of anxiety can be seen in the meanRR of 757-947 ms during the supine O_2 mask baseline (Table 8-7) compared to Resting 965-988 ms (Table 6-8); anxiety giving an increased heart rate from 61-62 bpm to 63-79 bpm.

These anxiety effects for some subjects may be the cause of the increased spread in the 95% confidence intervals for the baseline O_2 mask indices (Table 8-7) compared to Resting (Table 6-8). The indices known to be affected by

[14] Sufentanil is a synthetic opioid analgesic drug, about 5 to 10 times more potent than fentanyl.

anxiety are shown in the shaded area of Figure 9-1, normalised to Resting confidence intervals. Some other indices are shown; not all are affected by anxiety with increased confidence intervals.

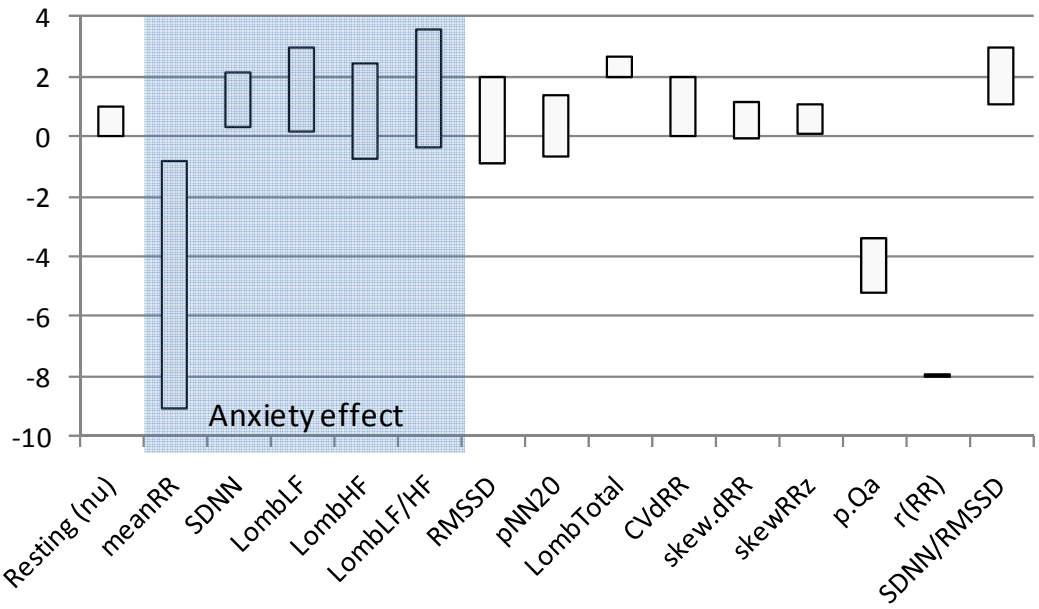


Figure 9-1 Anxiety effect on confidence interval range for O_2 mask baseline normalised to Resting. Indices known to be affected by anxiety are grouped together in the shaded area.

Some of the indices in this O_2 mask study have a similar response with sleep (**SDNN**, **r(RR)**, **SDNN/RMSSD**) and others with meditation (**LombTotal** and **pQc**). The differences may be caused by the magnitude of anxiety in the pre-surgical setting causing increased sympathetic activity.

9.2.6 Baseline - oxygen mask

The dual requirements for minimal intervention to the airway while maintaining subject safety required some respiratory intervention. All subjects received oxygen using a facemask from the start of the study to ensure adequate oxygenation after fentanyl administration. This avoided the use of interventions common in other studies such as paced breathing, intubation or mechanical ventilation [250, 252].

The oxygen mask has two effects on HRV that both increase vagal activity: the dead space of the mask, and use of 100% O_2.

Mask dead space

One study compared the use of a facemask to normal breathing without a facemask. Furutani *et al.* [248] applied a mask to subjects sitting on a bike ergometer and found the mask itself increased HFnu (i.e. increased vagal activity and decreased LF/HF). They suggested it was the deadspace of the mask (320 ml) that caused this effect.

Compared to no mask, Gole *et al.* [263] found a facemask with either air or 100% O_2 (45 min sitting, 15 br·min^{-1}) increased HFnu and decreased LFnu.

It is also known that a mask (deadspace 40-60 ml) [214] or respirator [215] will increase tidal volume and decrease respiratory frequency, and that a 50% increase in tidal volume would lead to a 15% increase in RSA [244] (which correlated here with SDNN). This study (with a mask deadspace of 172 ml) did not investigate these respiratory parameters, and effects of the mask on tidal volume may be lessened as respiratory depression development reduces the depth of breathing.

Application of 100% O_2

Some studies comparing mask and air to mask and O_2 found that O_2 increased vagal activity. (Note: This is not hyperoxia[15].)

Bartels *et al.* [219] showed an increase in vagal tone when comparing facemask with air (compressed) to facemask and supplemental 31% O_2 with increased HF, and decreased heart rate and LF/HF in patients with chronic obstructive pulmonary disease.

Three studies used 100% O_2 facemask with paced breathing at 15 br·min^{-1} and found increased vagal activity with increased HF and decreased LF/HF and heart rate whether sitting for 45-55 min [262, 263], or supine with 5 min each at 40, 70 and 100% O_2 [249].

The application of an O_2 facemask in this study, in conjunction with some anxiety, was associated with increased HR, **LombTotal**, and **SDNN/RMSSD**

[15] Hyperoxia is higher than normal oxygen tension, allowing O_2 to be dissolved in the blood e.g. by breathing air or oxygen at greater than atmospheric pressures such as in a hyperbaric chamber.

compared to Resting, with no significant effect seen on other time domain or spectral indices. For these indices, the baseline shift could mask the fentanyl effects.

9.2.7 Respiratory depression

The development of respiratory depression by definition is associated with decreased rate and depth of breathing which are known to affect HRV. Respiratory depression can lead to hypercapnia, and if severe to hypoxia which can also both affect HRV, though opioids significantly reduce the hypercapnic and hypoxic effects on ventilatory drive [6].

Decreased rate and volume of breathing

Poyhonen *et al.* [213] separated the effects of rate of breathing (12 and 8 br·min^{-1}), depth of breathing (± 20%), and $ETCO_2$ (4, 5 and 6%). During paced breathing (12 br·min^{-1}) decreased tidal volume caused decreased HF and HFnu (with no change to LF and LF/HF). Decreased rate of breathing from 12 to 8 br·min^{-1} shifted the power from HF into LF (as occurs in meditation) with increased LFnu and LF/HF during spontaneous ventilation (and this also occurred during mechanical ventilation and during anaesthesia).

This shift in breathing rate, that counteracts the expected fentanyl effects when measured with spectral analysis, may be one reason for contradictory results in fentanyl studies with results depending on the method of ventilation.

Hypercapnia

The $ETCO_2$ can be expected to increase 1-2 mmHg·min^{-1} with cessation of breathing. Two subjects (6, 10) were classified as hypercapnic ($ETCO_2$ > 45 mmHg) during the baseline but their $ETCO_2$ dropped after fentanyl administration. No subjects had hypercapnia in the period between fentanyl administration and the first central depression or the first critical respiratory depression event. Hypercapnia occurred only towards the end of the 5 min study for four of the subjects (3, 6, 8 and 10).

Hypoxia

Using an O_2 mask for the 10 min of this study ensured hypoxia did not occur. This was confirmed with SpO_2 remaining above 96% for the eight subjects with data.

9.2.8 Use of HRV over short term periods

The use of HRV over short 30 beat periods has only been validated in the earlier part of this study. Many of the selected indices have not been widely used outside studies by the original authors. Further validation over larger studies is required to confirm the usefulness of these indices and their application in 30-beat analyses.

Parasympathetic activity

The widely used indices of vagal tone (RMSSD and HF) have proven useful over periods as short as 10 s [210, 211] and have been used as prognostic indicators of cardiac mortality to replace longer monitoring over 5 min to 24 hr [316, 436] The total variability with SDNN has also been shown to be useful over 10 s [437].

Detection of other indices over short term

All other indices require periods longer than 10 s to give similar results to indices measured over 5 min. The development of multi-time analysis has shown the relative sympathetic indices (LF/HF and LFnu) can be measured in 50-70 s depending on the condition being investigated [450, 451]. This is half the recommended 2-5 min.

Earlier in this thesis (Chapter 6.5.5) it was shown that sympathetic spectral indices using Lomb-Scargle method were able to identify paradoxical shifts in power that occur during meditation over 30-beat periods (22-25 s) but this requires wider validation. The reduced total power seen in this study may limit the ability to detect the shift in power caused by slowing respiratory rates.

Other HRV indices

The survey of HRV indices did not include the plentiful nonlinear indices being applied to HRV analysis that reflect properties such as complexity and correlation rather than components of the autonomic system [142, 475-478].

These nonlinear indices may prove useful if they can be applied to very short-term windows.

9.2.9 Pre-existing conditions

Although subject histories were examined for pre-existing conditions that are known to affect HRV, the subjects did not undergo screening tests for cardiac autonomic function.

Inspection of the baseline data shows there is the possibility of autonomic imbalance in eight of the ten subjects studied with signs of: parasympathetic damage, parasympathetic hyperactivity and sympathetic hyperactivity.

Resting tachycardia with low SDNN (subjects 3 and 10 in Figure 9-2) can indicate parasympathetic damage common in cardiovascular autonomic neuropathy from diabetes [479] and may explain the lack of HRV response seen here, particularly in subject 10, with extended clinically observed UAWO.

Parasympathetic (or vagal) hyperactivity is not as common in the literature as other autonomic imbalances. It is affected by anorexia nervosa with increased SDNN (by > 40%) and HF, and decreased LF/HF [480, 481]. One subject in this study had a low baseline heart rate (subject 6) but better indicators for this are high SDNN, RMSSD, pNN20, (subjects 6, 8 and 9) with high HFnu and low LF/HF (only subjects 6 and 8). These two subjects showed a drop in HFnu with fentanyl; they were also the two youngest subjects in the study and thus are expected to have higher levels of HRV [482, 483].

Chronic sympathetic hyperactivity can be caused by many medical conditions, or psychosocial and behavioural conditions[16] [484]. Maule *et al.* [485] found ulcerative colitis to cause doubling of the LF/HF with almost 40% increase in LFnu and 33% decrease in HFnu. Piccirillo *et al.* [486] showed subjects with anxiety neurosis have an LF/HF four times that of normal subjects, but this was associated with decreases in LFnu, HFnu and total power. In congestive

[16] Medical conditions: obesity, insulin resistance or diabetes, hypertension, depression, anxiety, congestive heart failure, sleep apnoea.
Psychosocial and behavioural conditions: chronic stress, social isolation, hostility, smoking, sleep deprivation, stimulant abuse.

heart failure, Mortara *et al.* [487] found one group with sympathetic hyperactivity, high LF/HF and LFnu, and another group with no LF power and high HFnu.

Standard HRV is not the best measure of cardiac sympathetic outflow; attempts to find a better index of sympathetic activity have found nonlinear HRV indices may be more suitable. Baumert *et al.* [488] could not find a correlation between the standard HRV measures and the gold standard of cardiac sympathetic outflow, cardiac norepinephrine spillover, in subjects with major depressive disorder or panic disorder, but they found a moderate link with a nonlinear measure of complexity. Rachow *et al.* [489] used correlation with phasic sympathetic skin activity (electrodermal response) to find a nonlinear HRV index of sympathetic activity.

In this study, sympathetic hyperactivity may be indicated for four subjects (1, 5 (borderline), 7, and 9) by high LF/HF during the baseline with high LFnu and low HFnu (Figure 8-17 c, a, and b respectively).

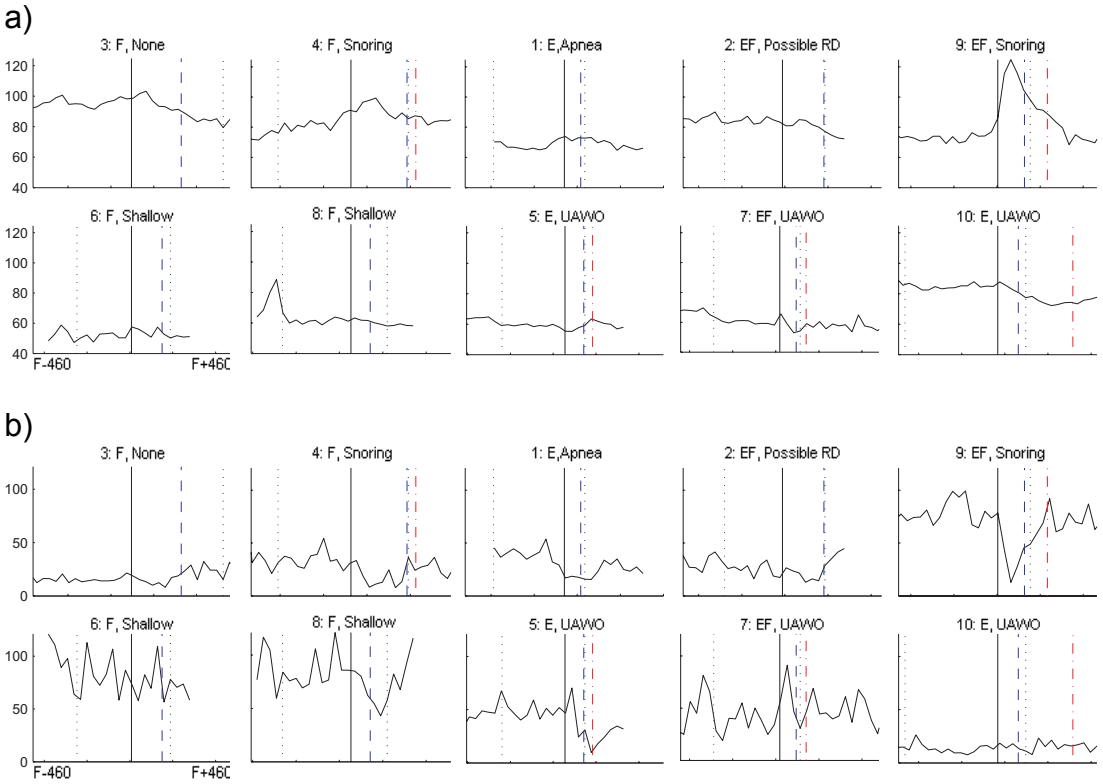


Figure 9-2 Autonomic imbalance identification with a) mean heart rate and b) SDNN

9.2.10 Summary fentanyl-induced changes on HRV

The overall effect of changes on impacting HRV is confusing (Table 9-1). During the baseline period, pre-surgical anxiety increases sympathetic and decreases vagal activity. This is counteracted by the use of an O_2 mask, the dead space and 100% O_2 both increasing vagal activity.

As respiratory depression develops after fentanyl administration, reduced depth and rate of breathing decreases vagal activity as does hypoxia; these decreases are offset by hypercapnia increasing vagal activity. In this study we can exclude the effects of hypoxia and hypercapnia. However, the slower rate of breathing by itself can also cause paradoxical increased sympathetic power though this will be limited as total power also decreases with fentanyl.

The largest impact on fentanyl-induced change of HRV is the underlying autonomic function of individual subjects.

Table 9-1 Summary of effects known to impact fentanyl results (this study shown in the central columns)

Index	Short term use	Anxious subjects	Mask dead space	100% O_2	O_2 mask baseline	Our result with fentanyl	Published with fentanyl	Low dose midazolam	Opioid with midazolam	Decreased rate of breathing
HRV, SDNN	Y	↑		-		(↓)	↓	↑		
RMSSD	Y					(↓)				
pNN50						(↓)				
SDNN/ RMSSD	Y				↑					
LF		↑		↑			↓-		↓	↑-
HF		↓	↑	↑			↓-		↓	↓
LF/HF		↑		↓			mx	-	↓	↑-
HFnu				↑			↑-		↑	↓
Lomb Total					↑	↓	↓-		↓	
Mean HR	Y	↑		↓	↑		↓-			

Notes: () trend, - no change, mx mixed results (↑↓-);
Abbreviation: Y, yes.

9.3 HRV preceding respiratory depression

The possibility that the traditional time or spectral indices for cardiac vagal activity or RSA could be effective in monitoring respiratory depression because of an association with opioid-induced loss of airway stability was not supported by this small pilot study with a failure to find any effect on vagal indices preceding critical respiratory depression, whether by upper airway obstruction or central depression.

Analysis of the peri-CD and peri-UAWO data showed: a) for most indices that showed a change, it was due to slight shifts in baseline levels that occasionally reached statistical significance, and b) only one short-term HRV index, **PolVar20**, had dynamic changes for some subjects that occurred after fentanyl administration and that dropped suddenly at the critical respiratory event, however these did not reach statistical significance.

9.3.1 PolVar20

The response of **PolVar20** (if baseline levels were not high) was to increase with fentanyl administration or with the approach of respiratory depression, and then suddenly decrease with the onset of respiratory depression. **PolVar20** in this situation may be acting as an indicator of brief or intermittent sympathetic activity.

This **PolVar20** response of intermittent bursts of sympathetic activity is similar to that described by Schirdewan in the 5-10 min preceding ventricular tachycardia [360, 490].

This transient low variability index derived from symbolic dynamics was first described in the HRV literature by Voss [357] identifying short phases of low variability with less than 20 ms between successive beat intervals and able to detect intermittent decreases in HRV. While **PolVar20** has been studied with cardiac conditions, increases identifying congestive heart failure and preceding ventricular tachycardia [360], it has not been studied under normal physiological conditions.

The oxygen mask had no significant effect on baseline **PolVar20** (compare O_2 mask baselines Table 8-7 to Resting in Table 6-8). Further work is needed to understand the **PolVar20** response to autonomic nervous system changes without opioids and modify the index for subjects with high resting levels of SDNN so it is independent on baseline HRV characteristics.

9.3.2 Statistics

Two problems became obvious with the statistical methodology used:

a) The use of confidence interval proportion of overlap to generate p-values limited the minimum p-value obtained for skewed distributions (i.e. where one confidence limit and the median are both zero).

b) Testing multiple indices over multiple consecutive periods reduced the likelihood of any index reaching statistical significance at any specific time point. Leaving visible those indices close to statistical significance provided information on indices likely to have sustained trends rather than occasional baseline shifts.

9.3.3 Endpoint determination

The detection of critical respiratory depression is delayed due to physical lags and the physiological process.

The physical lags include: 2.9 s for capnography gas sampling to occur in a 3 m tube with sampling delay and rise time [491]; and data processing within the physiological monitor.

With the decrease in autonomic activity that causes development of respiratory depression, the respirations decrease in volume and frequency. Eventually the next breath fails to appear; only by the absence of the “next breath” after a pre-determined time has passed is there certainty the event occurred. This is an indistinct event. There is a difficulty in using an indistinct event as the end-point when it occurs some time after the change in autonomic activity that causes it.

At various times, the occurrence of the indistinct respiratory depression event is able to be measured with $ETCO_2$, physiological monitor apnoea alarm, and eventually observable airway collapse. Using these measurable endpoints does not provide a consistent time to the change in autonomic activity that precedes it. Furthermore, with the development of respiratory depression, partial or complete airway collapse decreases the effectiveness of $ETCO_2$ [492].

The endpoint timing was further confounded by possible mask leak, and missing data collection. The missing data prevented the initial respiratory depression event from being identified in some subjects, however, given the indistinctness of the event being measured, and the individual subject differences in progressing through the events (mild CD to full respiratory depression), this was inconsequential.

9.3.4 Magnitude of respiratory depression

The likelihood, magnitude and duration of respiratory depression are increased by [6]: higher doses, age (longer elimination half life of opioids), concurrent administration of central nervous system depressants (alcohol, barbiturates and benzodiazepines), compromised pulmonary status (chronic obstructive pulmonary disease or heavy smoking), renal dysfunction (impaired drug excretion though this doesn't affect fentanyl clearance), opioid naivety, natural sleep and sleep-inducing drugs.

9.3.5 Effects of critical respiratory depression

Secondary effects during critical respiratory depression may impact on HRV: particularly hypercapnia, and hypoxia.

For this study we only examined the period up to central depression, a range of 76-259 beats after fentanyl administration (maximum 2:42 min), and critical respiratory events, 95-198 beats. Use of the oxygen facemask for the baseline 5 min ensured that subjects were not hypoxic (SpO_2 > 96%) within the study period. Hypoxia and hypercapnia can be excluded as causes of HRV changes in the peri-UAWO analysis.

9.3.6 Sleep apnoea

While we were not looking for a flurry of sympathetic activity, it is a common occurrence in literature on sleep apnoea, where subjects continually arouse to rectify their airway and respiration.

Sleep apnoea

Obstructive sleep apnoea is a disorder in which loss of pharyngeal dilator muscle tone at sleep onset causes recurrent pharyngeal collapse and temporary cessation of breathing (apnoea).

Over the course of an apnoea, a number of respiratory events occur: arterial oxygen tension (PaO_2) decreases, arterial carbon dioxide tension ($PaCO_2$) increases as a function of duration of apnoea, ventilatory effort progressively increases as the apnoea proceeds and intra-airway pressure becomes progressively more negative [34]. Each of these components has an impact leading to arousal from sleep.

Sleep apnoea arousal

At the end of each apnoea, asphyxia triggers a brief arousal from sleep that abruptly increases sympathetic activity, and suppresses vagal tone, precipitating surges in blood pressure and heart rate that restore pharyngeal patency [34, 120, 493, 494]. Repeated cycles of hypoxia and hypercapnia cause oscillations in both cardiac parasympathetic and sympathetic nervous activity [34, 495].

Sleep apnoea and anaesthesia

The underlying mechanisms leading to airway obstruction in sleep apnoea and during anaesthesia are similar; anaesthetic agents inhibit the respiratory activity of UAW muscles more than the diaphragm, creating a potential for narrowing or complete closure of the pharyngeal airway during anaesthesia [92].

It is not clear how much small or residual quantities of anaesthetic and sedative agents disturb the control of the airway. This topic is difficult to study because of the sleep state of the patient. When aroused, airway control may be satisfactory, but when left alone, the same patient may have persistent

airway obstruction or may show repeated cycles of obstruction and recovery [107].

Sleep apnoea arousals and HRV

Sleep apnoea studies with HRV have compared undisturbed sleep to the area around the end of the apnoea (based on return of airflow), 1 min before and 1 min after, and found increased LF power around apnoeas and hypopnoeas, irrespective of arousal visibility or type of respiratory event [496]. Analysis of overall night time HRV by Park *et al.* [497] found the number of apnoeas and hypopneas corresponded with LF/HF.

Freilich *et al.* [498] suggest an intact sympathetic system is a key component of the arousal-related increase in heart rate. They found in the 10 s following spontaneous arousals from sleep, healthy subjects had a large decrease in meanRR (90 ms) compared to autonomic failure patients (with sympathetic denervation) who had a reduced heart rate response (decrease in meanRR 40-50 ms).

In this study, the burst of sympathetic activity seen by some subjects occurs before the critical respiratory event, maybe as an attempt at arousal to restore patency of the upper airway. The lack of change in **PolVar20** for some subjects in this study may be indicative of decreased function of the sympathetic system.

Sympathetic hyperactivity

Apnoeic subjects have chronic diurnal elevation of adrenergic tone and they develop excessive cardiovascular sensitivity to sympathetic stimulation due to changes in the endothelial response [495].

Iturriaga *et al.* [499] simulated hypoxia from sleep apnoea in rodents (8 hr with 20 s of 5% O_2 for 14-21 days at a rate of 12 hr^{-1}). In the 5 min after a hypoxic event, the rodents had an enhanced reflex ventilatory response with increased LF/HF.

The large increases in **PolVar20** for some subjects in this study may be indicative of sympathetic hyperactivity that develops over time with chronic sleep apnoea.

9.3.7 Implications of pre-existing autonomic neuropathy

While parasympathetic damage makes the **PolVar20** response less obvious, other forms of autonomic neuropathy may be indicative of the likelihood of airway collapse. The four subjects with possible sympathetic hyperactivity all reached UAWO ($ETCO_2 = 0$), whereas the two subjects with parasympathetic dominance did not. Further investigation could determine whether parasympathetic hyperactivity was protective of airway patency, and sympathetic hyperactivity not protective.

9.3.8 Other uses for measuring autonomic neuropathy

The ability to perform HRV in a 30 beat window may lead to more effective use of HRV in the critical care environment.

HRV is being used in some critical care areas, particularly intensive care units and emergency care departments [500-502]. The HRV indices are being applied to prediction, early diagnosis and progression of critical conditions such as sepsis, septic shock, multiple organ dysfunction, severe brain injury and myocardial infarction. These are defined by autonomic cardiovascular abnormalities such as sympathetic hyperactivity or sympathetic dysfunction [503, 504].

Sympathetic overactivity causes tachycardia that has limited benefit in augmenting systemic blood flow but causes myocardial oxygen demand to outstrip supply and has major negative impacts on cardiac mortality [503, 505]. Inappropriate sympathetic activity is also implicated with the onset of septic shock, one of the commonest causes of death in intensive care units [506], and in multiple organ dysfunction [502]. Sympathetic activity is able to be monitored with spectral analysis, usually determined using FFT or AR methods over 2-5 min windows that require extensive processing making them unsuitable for regular daily use in these clinical areas [507]. Time domain measures SDNN and RMSSD are also being used over multiple 5 min blocks

to discriminate and monitor progression of these conditions [504] and predict trauma outcome within 12 hr of monitoring [508].

The techniques used in this thesis provide the possibility of monitoring these clinical conditions in a real time application. Further research on the use of short-term 30 beat HRV in these areas is recommended.

9.4 Summary of discussion

Short-term HRV of nonstationary data found, for some subjects, a short burst of cardiac sympathetic activity in the period after fentanyl administration and before a critical respiratory event. Sympathetic activity is a common occurrence during the arousal phase of sleep apnoea to restore airway patency.

The use of short-term 30 beat windows in this study is supported by wide use of 10 s SDNN and RMSSD but most of the other indices used here still need wider validation with short periods than that provided by this thesis.

The most likely reason for not detecting the enhanced parasympathetic ANS effects of fentanyl with the traditional time and spectral domain indices in this study is insufficient time in 5 min for the full fentanyl effect to occur. The trend to decreased HRV seen here may have been due to the full effect of fentanyl being lessened by the anxiety of subjects pre-operatively, by the concomitant low dose of midazolam, and use of a facemask.

The CNS-depressant effect of fentanyl, sedation with reduced rate and depth of breathing, may occur before cardiac parasympathetic or sympathetic effects occur or are identified with HRV. The slowing of respiratory rate has the effect of shifting power from HF to LF bands thus reducing the effectiveness of spectral indices to detect the expected opioid effect of increasing HF and decreasing LF power.

Using only the patient history for screening allowed enrolment of subjects with apparent imbalance of their autonomic function. This immediately highlights the main limitation of HRV for indirect measures of non-cardiac ANS function; subjects must have a functioning autonomic nervous system.

This leads to the possibility of using the methods of determining short-term HRV for monitoring autonomic neuropathy in critical care areas.

Chapter 10. Conclusion

This study started with a search for some premonitory indicator of opioid-induced respiratory depression, and in particular, airway collapse. The focus was on a possible link between cardiac vagal tone and the respiratory vagal tone that rhythmically strengthens airway tone during inspiration, to prevent airway collapse.

This study did not find enhanced cardiac vagal tone in response to fentanyl in < 5 min using 30 beat windows. Lomb-Scargle analysis, although shown to correctly report on power shifts in respiration with meditation, had no significant effect.

Although patient histories were examined, the subjects were not screened for cardiac autonomic function and this may have limited the ability of this study to find a link between opioid-induced airway collapse and HRV with eight of the ten subjects showing signs of autonomic imbalance.

For some subjects, PolVar20 detected a flurry of sympathetic activity before a respiratory event. This is likely to be an arousal attempt to restore patency to the airway.

The hypothesis of this thesis is supported by PolVar20, a short-term non-stationary measure of HRV that detected a change in sympathetic activity related to a shift in the airway stability.

The adoption of PolVar20 as an indicator of opioid-induced respiratory depression cannot be assumed as it is only able to detect this respiratory depression in a small subset of patients in the clinical setting; those not suffering from any cardiac autonomic neuropathy whether it is due to degeneration or hyperactivity.

10.1 Main findings overall

Survey and validation of short-term indices for 30-beat periods found:

- All RSA indices and most Poincaré plot indices (length, width and area) were strongly correlated to time domain measures (SDNN or RMSSD).
- All the selected indices were able to differentiate between Resting and at least one physiological state using 30 beat windows.
- Relative spectral power analysis (LFnu, HFnu and LF/HF) over 30-beat windows using a Lomb-Scargle algorithm is able to provide the same data as over 300 beat windows.
- Spectral indices using the Lomb-Scargle algorithm were able to correctly identify paradoxical shifts in power during meditation.

We found by studying fentanyl, a fast-acting opioid, over short time periods of 30 beats, that:

- Few indices respond to fentanyl within the 300 beat period of the study.
- Indices of vagal activity had nonsignificant changes.
- Some subjects had consistently large responses to many indices but unrelated to the extent of respiratory depression.
- Screening patient histories was not sufficient to exclude subjects with possible cardiac autonomic neuropathy.

In the period around critical respiratory events:

- PolVar20 identified a flurry of sympathetic activity that occurs with some (reactive) subjects in the period after fentanyl administration with a large drop concurrent with any respiratory depression event, whether it was a central depression or a short or long UAWO.
- Other indices did not respond consistently in the periods preceding central depression or critical respiratory depression.
- **PolVar20** is worthy of further investigation.

10.2 Considerations for future work

10.2.1 Use of short-term HRV

The literature survey identified indices that reflected parasympathetic and sympathetic activity and that could be used over short-term periods (< 1 min). The survey included indices from the traditional time domain, spectral domain, Poincaré plots, and other heart rate characteristics. Nonlinear indices not directly related to sympathetic and parasympathetic activity were not investigated here but should be included in any further studies.

10.2.2 Sleep apnoea

While we were not looking for an increase in sympathetic activity, it is in accordance with literature on sleep apnoea, where subjects arouse to rectify their airway and respiration.

This leads to a possible area of future work investigating the HRV indicators with sleep apnoea looking for evidence of the link preceding airway collapse. This study limited itself to the first critical respiratory event, but other effects will develop with the progression of respiratory depression without 100% O_2, particularly hypercapnia and hypoxia. The effect of these developments needs to be quantified in the HRV.

10.2.3 Defining the end-point

Better classification of the descent into respiratory depression, both the beginning and end of critical respiratory events, arousal, and return to respiration is needed to identify the specific HRV responses to these events.

The difficulty is in measuring the decrease in airway tone preceding collapse: spirometry and capnography occur after the airway collapse has occurred, have inherent delays due to gas sampling times and the signals are obscured by technical problems with mask leak.

Respiratory depression is not an on-off event, but is a waxing-waning process. As breathing slows and decreases in volume, at what point does breathing stop. It can only be identified by a lack of a “next breath” at the point where one may have been expected. With respiratory depression there is an

increasing gap between the time of the last known breath and the lack of a "next breath". It is unknown whether it is a temporary cessation of breathing or a complete stop until more time has passed.

10.2.4 Morphine

The techniques used in this study, with very short-term HRV over 30 beat windows, need to be tested with other opioids like morphine.

Morphine is the usual opioid given for post-operative pain relief with the subsequent occasional problem of respiratory depression [11, 24, 25]. Morphine is slow in onset with long lasting effect and does not allow rapid titration to effect. This leads to problems with its use in a study of respiratory depression: patients must be monitored for hours until a respiratory depression event; the respiratory event cannot be predicted and depends on many factors in the subject's environment and individual post-operative recovery; increasing the dose of morphine is not clinically acceptable and would not necessarily lead to a respiratory depression event; the respiratory depression needs confirmation by the attendance of a skilled clinician (anaesthetist); and the subject must be kept safe throughout this monitoring.

The short-term 30 beat HRV may not be the best method to monitor morphine-induced changes over longer periods. It is likely that the morphine, with its longer time to peak effect, would use a moving window for analysis, with non-stationarities expected as the morphine effect changed.

10.2.5 Index improvement

PolVar20 has not been widely investigated so the normal characteristics are unknown. Before being useful, **PolVar20** will need: a) to be improved so it is not reliant on SDNN, particularly for subjects with high baseline levels; b) to have wider testing to determine its range of application and ensure the range of application is not too narrow due to pre-existing conditions in post-operative patients that may confound the measurement (e.g. treatment for cardiac, blood pressure, diabetes or other autonomic nervous system diseases); and c) a change to the statistical measurement to suit the skewed distribution of this index.

10.2.6 Screening autonomic activity

If further study is done looking for a specific link between critical respiratory events and HRV, subjects will need to be screened to confirm cardiac autonomic function and to identify subjects with imbalances of the autonomic nervous system. Subjects likely to benefit from post-opioid monitoring in the clinical setting will likely have a large range in autonomic function and airway stability may be predicted by the type of autonomic neuropathy.

The large range in cardiac autonomic function is one of the biggest challenges in using HRV for any indirect measure of autonomic nervous system activity.

10.2.7 Critical care applications

The ability to perform HRV in a 30 beat window may lead to more effective use of HRV in the critical care environment.

HRV is being used in some critical care areas for prediction, early diagnosis and progression of critical conditions such as sepsis, septic shock, multiple organ dysfunction, severe brain injury and myocardial infarction. These are defined by autonomic cardiovascular abnormalities such as sympathetic hyperactivity or sympathetic dysfunction.

The techniques used in this thesis provide the possibility of monitoring these clinical conditions in a real time application. Further research on the use of short-term 30 beat HRV in these areas is recommended.