The effect of pain associated with delayed onset muscle soreness on the autonomic nervous system as measured by heart rate variability

Larissa Morgan

A dissertation submitted in partial fulfilment of the requirements for the Master of Osteopathy, Unitec Institute of Technology New Zealand, 2008
Acknowledgements

The following work represents a lengthy collaborative effort; therefore I’d first like to gratefully acknowledge my supervisors Robert Moran and Dr Craig Hilton for their contribution, guidance, and patient support in honing this project and bringing it to completion. Gratitude and recognition are also extended to all my colleagues and friends, who every week kept me going with feedback, counsel, unending cups of tea, and much needed comic relief. To my family, ‘thank you’ does not adequately express my appreciation for the unavering support and encouragement you have given me, which has in no small measure contributed to my achievements. Lastly, to Tui, who knew exactly what time to sit between me and the monitor – thank you for reminding me when to have a break.
# Table of Contents

ACKNOWLEDGEMENTS.................................................................................................................. 2

TABLE OF CONTENTS..................................................................................................................... 3

LIST OF FIGURES............................................................................................................................ 5

LIST OF TABLES............................................................................................................................... 6

ABBREVIATIONS............................................................................................................................. 7

INTRODUCTION............................................................................................................................... 8

  BACKGROUND............................................................................................................................... 8

  RESEARCH QUESTION................................................................................................................ 13

SECTION 1: LITERATURE REVIEW................................................................................................. 14

  CONCEPTS WITHIN OSTEOPATHY.............................................................................................. 14

  THE AUTONOMIC NERVOUS SYSTEM....................................................................................... 19

    Introduction............................................................................................................................. 19

    Influences on autonomic function......................................................................................... 22

    Indicators of autonomic function........................................................................................ 23

    Heart Rate Variability.............................................................................................................. 24

      Influences on heart rate variability..................................................................................... 25

      Measurement and parameters of heart rate variability....................................................... 28

      Limitations of heart rate variability as an indicator of ANS activity................................. 30

PAIN.................................................................................................................................................. 33

  Introduction................................................................................................................................. 33

  Physiological or sensory dimension of pain............................................................................. 33

  Affective or emotional dimension of pain............................................................................... 34

  The autonomic nervous system and pain................................................................................ 35

  Delayed onset muscle soreness............................................................................................... 37

    Physiology of delayed onset muscle soreness....................................................................... 37

    Delayed onset muscle soreness as a model of pain.............................................................. 40

SUMMARY AND AIMS OF RESEARCH......................................................................................... 42

REFERENCES.................................................................................................................................... 43

SECTION 2: MANUSCRIPT............................................................................................................... 56

  ABSTRACT.................................................................................................................................... 59

  INTRODUCTION............................................................................................................................ 60

  METHODS..................................................................................................................................... 62

    Design..................................................................................................................................... 62

    Subjects................................................................................................................................... 62

    Variables and Operational Definitions................................................................................. 63

      Independent variable............................................................................................................ 63

      Dependant variable............................................................................................................... 63

    Procedure................................................................................................................................ 64

    Intervention............................................................................................................................ 65

DATA ANALYSIS........................................................................................................................... 66

  Data extraction........................................................................................................................... 66

RESULTS......................................................................................................................................... 69

  Induction of pain associated with DOMS as measured by VAS.............................................. 70

  Normalised high frequency parameter of heart rate variability.............................................. 71

    Phase A – Baseline HF HRV data............................................................................................. 72

    Phase B – pre-DOMS, DOMS and recovery period HF HRV data........................................ 72

  Normalised low frequency parameter of heart rate variability.............................................. 75

    Phase A – Baseline LF HRV data............................................................................................. 75

    Phase B – pre-DOMS, DOMS and recovery period LF HRV data........................................ 75

DISCUSSION.................................................................................................................................... 79

  Outcomes................................................................................................................................... 79
## List of Figures

### SECTION 1: LITERATURE REVIEW

| FIGURE 1 | ORGANISATION OF THE NERVOUS SYSTEM. | 19 |
| FIGURE 2 | STRUCTURE OF THE AUTONOMIC NERVOUS SYSTEM. | 20 |
| FIGURE 3 | NORMAL ELECTROCARDIOGRAM WAVEFORM | 24 |

### SECTION 2: MANUSCRIPT

| FIGURE 1 | OVERVIEW OF STUDY DESIGN | 63 |
| FIGURE 2 | TYPICAL HEART RATE VARIABILITY ANALYSIS REPORT | 66 |
| FIGURE 3 | HIGH FREQUENCY HRV AND VAS VERSUS TIME | 73 |
| FIGURE 4 | LOW FREQUENCY HRV AND VAS VERSUS TIME | 76 |
List of Tables

TABLE 1  EXERCISE SUMMARY FOR ALL SUBJECTS--------------------------------- 65
TABLE 2  SUBJECT CHARACTERISTICS----------------------------------------- 69
## Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>AMI</td>
<td>Acute myocardial infarction</td>
</tr>
<tr>
<td>ANS</td>
<td>Autonomic nervous system</td>
</tr>
<tr>
<td>DOMS</td>
<td>Delayed onset muscle soreness</td>
</tr>
<tr>
<td>ECG</td>
<td>Electrocardiogram</td>
</tr>
<tr>
<td>HF</td>
<td>High frequency</td>
</tr>
<tr>
<td>HPA</td>
<td>Hypothalamic-pituitary-adrenal</td>
</tr>
<tr>
<td>HRV</td>
<td>Heart rate variability</td>
</tr>
<tr>
<td>LF</td>
<td>Low frequency</td>
</tr>
<tr>
<td>MTJ</td>
<td>Musculotendinous junction</td>
</tr>
<tr>
<td>PSD</td>
<td>Power spectral density</td>
</tr>
<tr>
<td>RSA</td>
<td>Respiratory sinus arrhythmia</td>
</tr>
<tr>
<td>SA</td>
<td>Sinoatrial</td>
</tr>
<tr>
<td>ULF</td>
<td>Ultra-low frequency</td>
</tr>
<tr>
<td>VLF</td>
<td>Very low frequency</td>
</tr>
</tbody>
</table>
Introduction

The Review of Literature commences with a background in which historical and contemporary concepts within the osteopathic profession are outlined, before the main body of the review which discusses the following topics: concepts within osteopathy; the autonomic nervous system; and pain.

Background

Spinal manipulation is a form of treatment practised in the manual therapy professions (e.g. chiropractic, osteopathy, and physiotherapy). Although not formally defined, many within the manual therapy professions would describe spinal manipulation as the application of a high-velocity low-amplitude force to the zygapophyseal joint of a specific spinal segment, which results in cavitation of that joint and is often accompanied by an audible ‘cracking’ or ‘popping’ sound (Gibbons & Tehan, 2006).

Within osteopathy there are an abundance of historical and contemporary anecdotal claims that spinal manipulation benefits health. Reported health benefits include reduced pain, greater freedom of movement, and overall improvement in function. Thus far, however, the physiological mechanisms of spinal manipulation have not been fully elucidated (see reviews by Evans (2002) and Pickar (2002)), and there is little evidence to support the effectiveness of spinal manipulation in any medical condition (see 2006 review by Ernst & Canter). Nonetheless, many osteopaths maintain the long held belief that the autonomic nervous system (ANS) provides one of the links\(^1\) between their manual therapeutic techniques, and many of the reported health benefits. The application of spinal manipulation and other techniques within osteopathy is partly based on a central tenet presented within many osteopathic texts, namely, that musculoskeletal or visceral disturbance can have effects elsewhere in the body via the ANS (DiGiovanna, Schiowitz, & Dowling, 2005; Kuchera & Kuchera, 1993; Parsons & Marcer, 2006; Stone, 1999).

\(^1\) Other claimed links between osteopathic manual techniques and reported health benefits include improved biomechanical efficiency, and improved blood supply and drainage.
The autonomic mechanisms which mediate interaction between musculoskeletal and visceral structures are usually named *autonomic reflexes*. Early researchers in this field focused on end-organ effects and as such the reflexes were described as *somato-visceral*, *viscero-somatic*, *viscero-visceral* and *somato-somatic* (Beacham & Perl, 1964; Sato & Schmidt, 1971). Although use of the word somatic varies between professional disciplines, here the term somatic refers to the musculoskeletal tissues of the body as distinct from viscera or some other entity such as the mind or the environment ("The new Collins dictionary and thesaurus," 1988). Within osteopathic literature the term ‘musculoskeletal’ is frequently interchanged with the term ‘somatic’ to describe the tissues of the human frame. Many viscero-visceral reflexes have been well investigated, for example the arterial baroreceptor reflex for blood pressure and the continence and micturition reflex of the urinary bladder (Janig & Habler, 1995). Less understood are the mechanisms responsible for viscero-somatic, somato-visceral, and somato-somatic reflexes (Bielefeldt & Gebhart, 2006). Yet, despite a rudimentary understanding, these reflexes and their purported clinical significance remain a fundamental concept within osteopathic texts and undergraduate education of osteopaths.

Within osteopathic theory, a visceral or somatic disturbance is said to perturb signals within local autonomic fibres. The signals from these fibres then converge onto the associated autonomic ganglia, many of which reside in close proximity to related vertebral segments. The aberrant input into the autonomic ganglia is purported to activate a reflex that alters signalling in autonomic fibres to other structures common to the same autonomic segment. Although not demonstrated in experimental studies, this osteopathic concept suggests that a low back dysfunction, for example, can affect lower abdominal and pelvic organ function through a somato-visceral reflex. Similarly, it has been claimed that poor respiratory function could manifest in segmental and palpatory changes in the mid-thoracic region through a viscero-somatic reflex (Kuchera & Kuchera, 1993; Parsons & Marcer, 2006).

---

2 Somatic: from the Greek Somatikós, meaning “of the body”. 1. of or relating to the soma: *somatic cells* (e.g. cells or tissue that resides outside the germline). 2. of or relating to an animal body or body wall as distinct from the viscera, limbs, and head. 3. of or relating to the human body as distinct from the mind: *a somatic disease* ("The new Collins dictionary and thesaurus," 1988).
As described by osteopaths, these concepts have lacked experimental support and have generated limited interest from researchers. A small number of clinical studies have shown that visceral disease may manifest in sensory, autonomic, and ‘skeletomotor’ changes (Beal, 1985; Bonica, 1992; Janig & Habler, 1995). Some clinicians have reported that visceral disease is accompanied by palpable musculoskeletal changes, and have further suggested that these changes may be useful for diagnosis and evaluation of treatment efficacy (Beal, 1983; Cox, Gorbis, Dick, Rogers, & Rogers, 1987; Nicholas et al., 1985).

More recently, an association between Type II diabetes mellitus and palpable skin texture change was reported (Licciardone, Fulda, Stoll, Gamber, & Cage, 2007). Licciardone et al. found the strongest and most consistent of the palpatory findings assessed (skin changes, trophic changes, tissue changes, tenderness, immobility), to be tissue changes on the right side of T11-L1 vertebral segments. Yet, many of the five palpatory elements assessed showed little difference between right and left, even though much of the pancreas is located left of the body’s midline. Furthermore, the most consistent skin texture changes were found at the level of T11-L1, a segmental level beyond the autonomic supply of the pancreas presented in many undergraduate physiology texts (T6-T10). Licciardone et al. speculated that “reflex viscerosomatic changes directly related to the progression of type 2 diabetes mellitus” might be responsible for the results, but could not eliminate the possibility of a spurious association or chance observation. Based on sparse and inconclusive literature, the link between visceral disease and musculoskeletal changes is not a clear and causal one, and could be considered associative at best. Moreover, if frank pathology does not manifest in consistent or clearly observable musculoskeletal changes, the osteopathic concept that non-pathological disturbance may result in similar change is less plausible.

Although the causality between visceral disease and musculoskeletal change is unclear, there is evidence of an interaction between visceral and musculoskeletal structures. The referred pain phenomenon is the most clinically recognised and investigated interaction between visceral and somatic tissues. The International Association for the Study of Pain describes referred pain “in neurological terms as pain perceived as arising or occurring in a region of the body innervated by nerves or
branches of nerves other than those that innervate the actual source of pain”, or in clinical terms “as pain perceived as occurring in a region of the body topographically distinct from the region in which the actual source of pain is located” (Merskey & Bogduk, 1994, p. 12).

Referred pain is not an autonomic reflex although may be associated with such reflexes. As a phenomenon, referred pain has long been recognised, with descriptions dating to the late 19th century (Ross, 1888, Ruch, 1961, & Sturge, 1883, as cited in Arendt-Nielsen & Svensson, 2001). Several theoretical models have been developed to explain the occurrence of referred pain, with the current and most accepted model being the convergence-projection theory (Arendt-Nielsen & Svensson, 2001). This theory is broadly similar to the osteopathic concept regarding autonomic reflexes in that the proposed mechanism is one of convergence. Although not wholly understood, referred pain is thought to occur by the following means: pain signals from injured tissue and normal sensory signals from other tissues converge within the dorsal horn of common spinal segments, however, in the barrage of information, the spinal cord cannot clearly distinguish the origin of the pain signal, and so a non-injured area is ‘perceived’ to be painful (Arendt-Nielsen, Laursen, & Drewes, 2000; Arendt-Nielsen & Svensson, 2001; Giamberardino, Affaitati, Lerza, & Vecchiet, 2000). Due to the clinical significance of referred pain (e.g. myocardial ischemia and pain in the left arm), this phenomenon is the most widely acknowledged and studied interaction between somatic and visceral structures.

In contrast to the referred pain phenomenon, the concept that somatic and visceral disturbance can influence other structures though the ANS has limited scientific support. With few human studies available, osteopathic literature has often relied on evidence from experiments on laboratory animals. Conclusions from these experiments, which are cited frequently and with little critique in osteopathic literature, are that stimulation of visceral and somatic nerves produce responses in cardiac ANS activity (Terui & Koizumi, 1984), and that noxious or painful musculoskeletal stimuli clearly and consistently modulate ANS activity and sometimes visceral function (Adachi, Meguro, Sato, & Sato, 1990; Kaufman, Sato, Sato, & Sugimoto, 1977; Sato, Sato, & Schmidt, 1997; Sato & Schmidt, 1987). Many of the same investigators have also found that innocuous mechanical stimuli affect the
ANS, albeit in a milder and less consistent manner than noxious stimuli (Sato et al., 1997; Sato & Swenson, 1984). Yet, within osteopathic literature, the findings regarding innocuous stimulation on the ANS are cited infrequently, potentially exposing a bias towards citing scientific data that supports osteopathic concepts.

More recently, osteopathy and other manual therapies have begun to formally investigate whether manual therapeutic techniques influence the ANS. Evidence has emerged showing that spinal manipulation and innocuous non-spinal stimulation affect autonomic activity (Budgell & Hirano, 2001; Budgell & Polus, 2006; Cooper, 2006; Driscoll & Hall, 2000; Eingorn & Muhs, 1999; Knutson, 2001; McGuiness, Vicenzino, & Wright, 1997; Zhang, Dean, Nosco, Strathopulos, & Floros, 2006); however, clinical outcomes have not been included in these studies. To date, studies have focused on the cause-effect relationship between manual therapy techniques and the ANS without regard to magnitude of effect, and whether such effect is clinically favourable or unfavourable. Furthermore, the investigations have not ascertained whether techniques that are purported to modulate the ANS are a necessary, or clinically relevant, part of care. The issue of clinical relevance is further weakened by insufficient critical review of the findings: any ANS effects observed in these investigations could be epiphenomena and not part of the causal chain purported to exist between musculoskeletal disturbance and effects elsewhere in the body.

On balance, the osteopathic concept that musculoskeletal disturbance can perturb the ANS is lacking support. The poorly elucidated mechanisms, inadequate appraisal of clinical relevance, and limited scope of research places the concept in a contentious light – despite associations suggested by current literature (Licciardone et al., 2007). It comes as no surprise then, as Stone (1999, p. 75) indicates in a contemporary osteopathic text, that the concept of ‘musculoskeletal irritation causing visceral disturbance’ is not a recognised clinical entity. Yet, within osteopathic textbooks and in oral history imparted in undergraduate training, the concept remains fundamental. The osteopathic profession maintains the long held position that manual therapeutic techniques favourably influence the ANS and are an appropriate part of care.

Dogma, coupled with limited and often inconclusive evidence, has done little to avert any controversy surrounding the concept that somatic or visceral disturbance affects
the ANS. As such, several questions remain as it is yet to be established whether a fundamental premise of osteopathic patient management has any basis: namely, does ANS perturbation from mild to moderate musculoskeletal disturbance/injury occur? Furthermore, the question is raised of whether it necessarily follows that such ANS disturbance will have unfavourable clinical effects. If osteopathic techniques do modulate ANS activity, can the techniques evoke an ANS response beyond any localised or short-term general effects, to ameliorate the so-claimed effects further afield? Although the impact of acute soft tissue injury on the ANS has been recently investigated (Grimm, Cunningham, & Burke, 2005), there is a scant amount of literature addressing these questions. There have been calls in the literature for a definitive illustration of whether mild to moderate musculoskeletal disturbance could have a deleterious impact on the ANS, and potentially on health and wellness. Establishing the nature and significance of this link is important to not only evaluate tenets within osteopathy, but may be of clinical relevance to those with acute injury or those in chronic pain states.

**Research question**

The aim of this pilot study was to explore whether delayed-onset muscle soreness (DOMS), as a model of pain and mild musculoskeletal injury, would affect the ANS as measured by heart rate variability (HRV). The rationale for employing DOMS was that it is simple to induce, non-invasive, and would simulate a soft tissue injury without involving gross trauma or heterogeneity of damage.\(^3\) Heart rate variability has been employed in a variety of fields to evaluate changes to the ANS, and although not a direct measure of mean autonomic tone, HRV was considered a well supported measure for this project.

---

\(^3\) Heterogeneity is used here to denote damage involving tissue types other than myofascia, such as bone, ligament, cartilage, nerves, and blood vessels.
Section 1: Literature Review

Concepts within osteopathy

Within osteopathy, the terms ‘philosophy’, ‘principle’, and ‘hypothesis’ are often used in reference to the ideas put forward by founding members of the profession. Based on review of osteopathic literature and dictionary definitions, the term ‘concept’ will be used to describe osteopathic ideas, and the term ‘tenet’ to denote a belief held in common by members of the osteopathic profession.

Within osteopathy, the approach to treatment is guided by several concepts, such as:

- The body is a unit
- Structure and function are reciprocally interrelated
- The body possesses self-regulatory, repair, and defence mechanisms
- The flow of body fluids is essential to health


Although these concepts are not unique to osteopathy, they help form the basis of the osteopathic perspective on health and disease.

Health, illness, and disease are concepts that differ between cultures and historically have been rich with theory and debate. Osteopathic texts regard health and disease as an interplay and balance between the emotional, chemical, and physical environments (DeGiovannna et al., 2005, pp. 10-15; Kuchera & Kuchera, 1993, pp. 7-12; Stone, 1999, pp. 15-27). The notion within osteopathy is that failure to adapt, or exposure to overwhelming external factors, can upset the balance between these environments and can prevail over the body’s capacity for self-maintenance (DeGiovannna et al., 2005; Stone, 1999). As a form of manual medicine, osteopathy emphasises the role the neuromusculoskeletal system plays in the manifestation of health and disease (DeGiovannna et al., 2005).

The concepts of ‘normal function’ and ‘dysfunction’ are commonly used within the osteopathic profession. Yet, these terms are hampered in a similar manner as that of health and disease. Stone (1999, pp. 28-56) has reasoned that disease may be
represented by a breakdown of communication between parts, and states that anything interfering with cellular signalling efficiency may disrupt the ability to adapt. Within osteopathy, this disruption is proposed to create a tendency towards disequilibrium, disease, and dysfunction. Stone’s dialogue reflects content found in other osteopathic texts (DiGiovanna et al., 2005; Kuchera & Kuchera, 1993; Ward, 2003), which assert that neural, fluidic, or mechanical barriers to physiological communication can influence the structure-function relationship. Thus, a general aim of osteopathic treatment is to remove tissue barriers and allow “better communication between parts to re-establish itself” (Stone, 1999, p. 32), in order to restore or maintain ‘normal’ function (Kuchera & Kuchera, 1993; Nelson, 2007b).

Correcting barriers to ‘appropriate communication’ provides one of the rationales for therapeutic techniques such as spinal manipulation. In a general sense, osteopaths claim to address perceived aberration in neural processing by treating parts of the body along the neural pathway that could be contributing to the dysfunction. The concept of addressing aberrations in neural processing is based, in part, on the structural distribution, widespread function, and anatomical relationships of the ANS. The ANS is distributed throughout the entire nervous system and has the essential function of maintaining homeostasis in conjunction with the respiratory, endocrine, and immune systems. Anatomically, fibres of the sympathetic division of the ANS are in close proximity to vertebral segments and associated soft tissues (muscle, tendon, and ligament). Fibres of the sympathetic division of the ANS exit the spinal cord between the first thoracic and second lumbar vertebra and are linked by a chain of ganglia extending the length of the thoracic spine lateral to the same segments.

Theorists within osteopathy surmise that the anatomical arrangement of the ANS, particularly the sympathetic division, makes the ANS accessible to osteopathic manual treatment. For example, within osteopathy, the perceived accessibility of sympathetic division permits the ANS to be influenced, albeit indirectly, with techniques such as spinal manipulation. Although spinal manipulation has, in recent times, been shown to have an effect on the ANS the mechanisms remain unclear (Pickar, 2002; Willard, 2007; Wright, 1995). Nonetheless, many osteopaths maintain the view that ‘balance’ and integration of ANS subdivisions has a significant role in health and disease, (DiGiovanna et al., 2005; Stone, 1999). As such, disturbance of a
body structure is considered by osteopaths to potentially perturb the balance between ANS subdivisions, or lead to an aberration in autonomic neural processing, which may influence other body structures with the same autonomic supply. This process is succinctly described in a text by Parsons and Marcer (2006), who state that “if a spinal segment or any of the structures supplied by it are disturbed, convergence into the common segment generates a reflex that may induce changes in any or all of the structures supplied by that segment”. However, the empirical consequences of this process have not been fully drawn out and tested, such as whether the effects of these autonomic reflexes can be objectively measured.

The functions of the musculoskeletal system are commonly regarded as being locomotion, defence, support, and communication. The musculoskeletal, or somatic, component of the body is considered by many osteopaths to be “the final common pathway by which we carry out our lives” (Stone, 1999, p. 96), a concept echoed in many contemporary osteopathic texts (DiGiovanna et al., 2005; Kuchera & Kuchera, 1993; Nelson, 2007a; Parsons & Marcer, 2006). Based on this view, osteopaths have developed the concept of somatic dysfunction to denote alterations in the body as a result of autonomic reflex mechanisms. From the Glossary of Osteopathic Terminology the accepted definition of somatic dysfunction is “an impaired or altered function of related components of the somatic (body framework) system: skeletal, arthrodial, and myofascial structures, and related vascular, lymphatic, and neural elements” (Glossary of Osteopathic Terminology, 2004).

The diagnosis of somatic dysfunction is based on subjective palpatory findings and patient feedback. Anecdotal evidence suggests that patterns of somatic dysfunction appear to follow ANS distribution although there is little scientific evidence to support this notion. Somatic dysfunction is considered to contribute to the effect of pathology, or be associated with pathology, however, osteopathic literature clearly distinguishes somatic dysfunction from organic pathology and maintains that trauma,  

---

4 Acute somatic dysfunction: Immediate or short-term impairment or altered function of related components of the somatic (body-framework) system; characterised in early stages by vasodilation, oedema, tenderness, pain and tissue contraction
Chronic somatic dysfunction: Impairment or altered function of related components of the somatic (body-framework) system, characterised by tenderness, itching, fibrosis, paraesthesia, tissue contraction
Primary somatic dysfunction: 1) The somatic dysfunction that maintains a total pattern of dysfunction. 2) The initial or first somatic dysfunction to appear temporally.
Secondary somatic dysfunction: somatic dysfunction arising either from mechanical or neurophysiologic response subsequent to or as a consequence of other aetiologies (Glossary of Osteopathic Terminology, 2004)
degeneration, and inflammation are not considered somatic dysfunctions (DiGiovanna et al., 2005, p. 16; Nelson, 2007a, pp. 12-13).

In order to ‘ameliorate’ somatic dysfunction, one of the aims of osteopathic treatment is to correct any interference the musculoskeletal system may have on autonomic function (DiGiovanna et al., 2005; Kuchera & Kuchera, 1993; Parsons & Marcer, 2006; Stone, 1999). The supposition of a cause and effect relationship between the ANS and musculoskeletal structures forms part of the rationale for the application of spinal manipulation techniques to treat somatic dysfunction. Osteopath author Fred Mitchell, Sr. illustrates this view when he noted that “implicit in the term somatic dysfunction is the notion that manipulation is appropriate, effective, and sufficient treatment for it” (Mitchell Jr., 1979), although none of these implicit assumptions (i.e. suitability, efficacy, and adequacy) have been objectively demonstrated to date.

Osteopathic literature has begun to gather evidence on whether manual therapy techniques such as spinal manipulation exert an influence on autonomic activity. Several studies within both chiropractic and osteopathic literature have evaluated spinal manipulation using a variety of outcome measures, including pain and ANS indices. Researchers have found excitatory effects, such as changes in heart rate, blood pressure, and respiratory rate (Knutson, 2001; McGuiness et al., 1997), altered heart rate variability (Budgell & Polus, 2006), increased sudomotor activity (Simon, Vicenzino, & Wright, 1997; Sterling, Jull, & Wright, 2001), increased skin temperature (Sterling et al., 2001), altered cutaneous blood flow (Karason & Drysdale, 2003), and post-manipulation hypoalgesia (Vicenzino, Collins, Benson, & Wright, 1998). Different speed of manipulation has also demonstrated an effect on the ANS (Chiu & Wright, 1996).

The ANS is also affected by non-manipulative stimulation of spinal segments, with Sato and Swenson (1984) observing that ANS responses persisted beyond the immediate post-application period (Budgell & Hirano, 2001; Fujimoto, Budgell, Uchida, Suzuki, & Meguro, 1999; Moulson & Watson, 2006; Sato & Swenson, 1984; Vicenzino, Cartwright, Collins, & Wright, 1998). Innocuous non-spinal stimulation has also been documented to cause various autonomic changes (Cooper, 2006; Simon et al., 1997). Despite these findings, however, the physiological processes to which
such effects may be attributed remain inconclusive. The diverging results seen in the literature has prompted Pickar (2002) to suggest that “more than one mechanism likely explains the effects of spinal manipulation”, and theories continue to abound regarding what therapeutic processes or physiological mechanisms are involved (Wright, 1995). Nonetheless, the effect of spinal manipulation on the ANS comprises only one aspect within the concept that musculoskeletal or visceral disturbance can perturb the ANS. Other aspects, such as whether ANS perturbation does occur and can be objectively measured, or indeed is clinically relevant, have not been adequately investigated.

In summary, a central tenet within osteopathy is that disturbance of a body structure may perturb the ANS. Perturbation to the ANS is thought to influence other body structures via common autonomic segments, and may result in somatic dysfunction. The source of the autonomic perturbation, plus any subsequent effects on other structures, is then thought to be amenable to manual therapy techniques such as spinal manipulation. Scientific evidence to support this tenet, the purported processes involved, and the manual therapy techniques used, is limited or inconclusive. Yet, despite this, osteopaths maintain their position that the ‘balance’ of the ANS is significant to health, and that manual therapeutic techniques to address ANS ‘imbalance’ are appropriate and effective.
The Autonomic Nervous System

Introduction

The autonomic nervous system (ANS) is distributed throughout the central and peripheral nervous systems (Figure 1). The ANS exerts continuous control over cardiac muscle, smooth muscle, glandular function, and temperature regulation. In conjunction with the respiratory and endocrine systems, the ANS brings about fine internal adjustments necessary for homeostasis.

![Organisation of the Nervous System](Image)

**Figure 1** Organisation of the Nervous System.
Adapted from Marieb (2001).

Anatomy and physiology texts differentiate the sympathetic and parasympathetic divisions of the ANS by anatomical, physiological, and neurotransmitter differences. These divisions are thought to function in concert, and are described in undergraduate texts as having opposite and antagonistic actions. Physiological effects of the sympathetic system are generally simplified as being arousing and are commonly described as the *fight, flight, or fright* responses. In contrast, the parasympathetic system has been described as *rest and digest*, as it promotes conservation of energy and regulates digestion. A third division, the enteric nervous system, regulates
sequential action of the gastrointestinal tract, although physiologists often consider this system separate as it is mostly governed by parasympathetic and sympathetic activity.

Texts describe both divisions of the ANS as displaying segmental organisation in the innervation of the body (Figure 2). In the sympathetic nervous system, fibres exit the spinal cord between the first thoracic and second lumbar vertebra. The fibres then pass to the sympathetic trunk ganglia flanking these vertebrae and follow one of three courses: synapse immediately at the same level; travel within the sympathetic trunk to synapse at a higher or lower level; or pass through the sympathetic trunk to synapse with prevertebral ganglia. In contrast, the parasympathetic nervous system has a more restricted distribution. Fibres originate from cell bodies within the brainstem and the second to fourth sacral segments of the spinal cord to synapse close to the target organ. The vagus nerve in particular is central to regulation of visceral organs as numerous vagal branches provide much of the parasympathetic innervation of thoracic and abdominal organs.

**Figure 2** Structure of the Autonomic Nervous System. Adapted from Snell (2001).
To maintain homeostasis, the ANS detects and responds to a variety of stimuli in the internal and external environment. This activity results in continual moment-to-moment responses and is commonly thought to be regulated by higher centre control and peripheral reflex arcs. Yet, as Janig and McLachlan (1992; 1999) observe, the diversity of the ANS is not limited to a simple neuroendocrine role, as discrete functional pathways and differentiation of reflex patterns have been identified at molecular, cellular, and integrative levels. The complex neuroendocrine role of the ANS is reflected in specialisation of the vagus nerve of the parasympathetic system. Berthoud and Neuhuber (2000) describe the vagus as having a peripheral and central interface: mechanical, chemical, and temperature receptors are found in and around organs, and the vagus also projects to the brainstem and forebrain. Berthoud and Neuhuber go on to discuss evidence regarding a vagal role in pain perception, in both a sensory and affective capacity, and that the anatomical connections of the vagus imply a potential for vagal signals to affect the entire organism.

Autonomic control of viscera is widely regarded as encompassing alteration in blood flow, blood glucose, motility, and secretions. Under stable environmental conditions, the regulation of visceral function occurs automatically at the organ level, without involvement of higher centres. The integration of visceral function frequently involves higher centres, and is reflected in the precise adaptation of certain organs to behavioural requirements, for example, bladder and bowel control and social context. These intricate processes are closely related to the nervous system, the endocrine system, autonomic reflexes, visceral sensations, emotion generation, and higher centre function (Janig & Habler, 1995).

In the cardiovascular system, the ANS is essential to cardiovascular control mechanisms, such as beat-by-beat fluctuations in heart rate and blood pressure. The sinoatrial (SA) node, which is the main determinate of heart rate, is richly innervated with sympathetic and parasympathetic fibres. Arousal states such as fear and excitement increase sympathetic activity to the SA node elevating heart rate and force of contraction. Sympathetic arousal also stimulates release of the hormones adrenaline and noradrenalin, and activation of the renin-angiotensin-aldosterone system of the kidney. These mechanisms act to alter blood pressure and sequester blood away from areas less vital when faced with threat (e.g. digestive organs).
Parasympathetic activity to the heart, mediated by the vagus nerve, produces states of relaxation and calm. Increased vagal activity to the sinoatrial node reduces heart rate and blood pressure, increases the beat-to-beat variability of the heart, and reduces force of contraction. Vagal governance of respiration also modulates cardiovascular function via respiratory depth and frequency (Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996). Vagal activity therefore regulates cardiovascular function through a direct contact with the SA node, and modulates heart rate and blood pressure through respiration (Stauss, 2003).

Failure or imbalance of the cardiac autonomic mechanisms is widely recognised to promote life-threatening cardiovascular events. Complex phenomena such as ventricular tachyarrhythmias and sudden cardiac death are the leading causes of cardiovascular mortality and are associated with increased sympathetic and decreased vagal tone. In people with prior cardiac injury, increased sympathetic and decreased vagal activity can significantly modulate the activity of cardiac pacemaker and conduction tissue, and unfavourably alter haemodynamics (Sztajzel, 2004; Zhong, Jan, Ju, & Chon, 2006).

**Influences on autonomic function**

The ANS is widely accepted to be influenced by numerous extrinsic and intrinsic factors, which can produce local or generalised effects. Extrinsic stimuli affecting the ANS range from physical exercise, postural change, extremes of temperature (heat and cold), pain, and mental stress. These stimuli can cause changes to blood flow and heart rate, and activation of hormones such as adrenaline and cortisol. Appraisal of danger, evident in the so-called *fight, flight or fright* response, is observed to have an immediate influence on the ANS – heart rate and blood pressure increase virtually instantaneously and are accompanied by sweating and pupillary dilation (Brooks, Koizumi, & Sato, 1979).
Numerous intrinsic factors, such as circadian rhythms and metabolic products, are recognised to have a widespread influence on the ANS. Extensive and often detrimental influences on the ANS are associated with many pathologies, such as endocrine, renal, and neurological disorders (Mathias & Bannister, 1999). Widespread effects on the ANS are also linked with aging (Docherty, 2002) and in consumption of chemical agents such as pharmaceuticals, alcohol, and recreational drugs (Brooks et al., 1979).

Although much knowledge regarding the ANS has unfolded in the last century, many questions still remain. Berthoud and Neuhuber (2000) comment that the mechanisms behind central regulation of ANS outflow patterns requires further exploration. Within the manual therapy professions, questions have predominately focused on whether autonomic activity is influenced by the mechanical stimulation provided by manual therapy techniques.

**Indicators of autonomic function**

Literature indicates that a variety of measures may be used to quantify changes in autonomic function. These include pupillary diameter (Briggs & Boone, 1988), speed of pupillary reflex (Gibbons, Gosling, & Holmes, 2000), skin sympathetic nerve activity (Ray & Monahan, 2002), muscle sympathetic nerve activity (Hume & Ray, 1999), distal skin temperature (Harris & Wagnon, 1987), skin conductance (Chiu & Wright, 1996; Petersen, Vicenzino, & Wright, 1993), plasma and salivary concentration of hormones and other neurochemicals (Costa, Lavin, Robertson, & Biaggioni, 1995; Tuchin, 1998), immune system activity (Brennan et al., 1991), and heart rate and blood pressure (Fujimoto et al., 1999). Although a number of physiological measures are available to quantify changes in the ANS, most are not direct measures of autonomic activity.

The status of the ANS can be directly assessed with scintigraphic and cardiovascular reflex tests. As these types of tests are invasive and not widely available, heart rate variability (HRV) has emerged as a simple non-invasive technique which evaluates
sympathetic and parasympathetic activity at the sinoatrial level (Sztajzel, 2004; Task Force, 1996).

**Heart Rate Variability**

Heart rate variability (HRV) measures the variation in heart rate between consecutive beats (Niskanen, Tarvainen, Ranta-Aho, & Karjalainen, 2004). The R-R interval of the electrocardiogram (ECG) (Figure 3) denotes the peak of the R-wave as the reference point to determine the duration between two consecutive beats. The variability of R-R intervals has been used for many years to evaluate the ANS in short term experiments in psychology, cardiovascular physiology, and pharmacology (Berntson et al., 1997).

Variation in the R-R interval represents the subtle regulation of the dynamic cardiovascular control mechanisms by the ANS. The sympathetic and parasympathetic activity directed to the sinoatrial node is mostly synchronous with each cardiac cycle, but can be modulated by central and peripheral oscillators such as the respiratory centre in the brainstem, or arterial baroreceptors (Task Force, 1996). The modulations cause a rhythmic fluctuation in sympathetic and parasympathetic discharge which manifests as short and long-term oscillations in the cardiac contraction period. Analysis of these oscillations may permit inferences to be made on the state and function of sympathetic and parasympathetic activity, the central oscillators, humoral factors, and the sinoatrial node itself (Task Force, 1996).

![Figure 3 Normal Electrocardiogram Waveform]("Electrocardiogram (ECG)," 2006).
The clinical utility of HRV is reflected in numerous published works on HRV in the context of cardiovascular physiology and medicine (Berntson et al., 1997). Reduced HRV is strongly associated with adverse outcomes (Dekker et al., 2000; Dekker et al., 1997; Liao et al., 1997; Tsuji et al., 1996), and HRV data has demonstrated that sympathetic activity is elevated in heart failure and coronary artery disease, and plays a role in hypertension (Eisenhofer et al., 1996; Huikuri et al., 1996; McCance, Thompson, & Forfar, 1993). Reduced HRV has been shown to be of prognostic relevance in progressive heart failure and is associated with increased mortality after acute myocardial infarction (La Rovere, Bigger, Marcus, Mortara, & Schwartz, 1998; La Rovere et al., 2003; Ponikowski et al., 1997). Consequently, the most common clinical application of HRV is predicting risk of death in patients with cardiac disease (Bigger et al., 1995).

The promise showed by HRV as an easy-to-use marker of cardiac autonomic activity increased its popularity of use. However, the significance and complexity of HRV was not widely appreciated or understood prior to the mid-1990’s. Consequently, the Task Force by European Society of Cardiology and the North American Society of Pacing and Electrophysiology was formed. The Task Force standardised methods of measurement, nomenclature, definitions, and described clinical applications of HRV (Task Force, 1996).

**Influences on heart rate variability**

To better understand the multiple influences on HRV and cardiovascular physiology, clinicians and investigators have drawn on non-linear dynamics or ‘chaos theory’. Although the term ‘chaos theory’ has yet to be formally defined, chaos can be more easily understood by describing it in terms of its behaviour. Chaos is believed by theorists to display characteristics of both randomness and periodicity, but is distinct from both. Put simply, random behaviour is unpredictable and never repeats itself, whereas periodic behaviour is predictable and repeats over a finite period of time. Chaotic behaviour is considered by theorists to appear disorganised (i.e. random) but is actually periodic (Decroly & Goldbeter, 1982; Goldberger, 1996; Kaplan & Yorke, 1979).
Chaos theory has been utilised to understand a variety of different processes. These range from fluid turbulence (Kaplan & Yorke, 1979) and weather (Lorenz, 1963; Pool, 1989b), to biological processes such as epidemics (Olsen & Degn, 1985; Pool, 1989a), biochemical reactions (Decroly & Goldbeter, 1982, 1985), and cardiac behaviour (Goldberger, Findley, Blackburn, & Mandell, 1984; Goldberger, Shabetai, Bhargava, West, & Mandell, 1984). To illustrate the characteristics of chaotic behaviour, that is, periodic from one viewpoint yet random from another, Denton, Diamond, Helfant, Kahn, & Karaqueuzian (1990) offer the following examples: the average behaviour of a container of gas molecules can be predicted, however the individual behaviour of a single molecule cannot; likewise, the average heart rate of a patient can be predicted, however the individual pattern of R-R intervals cannot.

The HRV data from a patient with heart failure shows reduced or restricted variability of the R-R intervals (Goldberger, 1997; Goldberger, Findley, Blackburn, & Mandell, 1984). Restricted variability is one that is more uniform and periodic, and shows predictable oscillations in the heart rate. In contrast, the HRV from a healthy heart demonstrates a variability which is more complex, less uniform, and more irregular. The complexity of the variation seen in healthy subjects has lead to the suggestion that a healthy cardiovascular system is one that can generate chaos (Denton et al., 1990). Research by Goldberger, who draws heavily on chaos theory, has in the past three decades provided insight into cardiovascular physiology and disease. Goldberger (1996) has remarked that a healthy heart beat is chaotic and states that “This kind of complex variability, rather than a regular homeostatic steady state, appears to define the free-running function of many biological systems”. A loss of complex variability in the cardiovascular system is proposed to be a common feature of cardiovascular disease. Such loss of complexity, that is, a more predictable and less chaotic state, means less adaptive capacity in a dynamic environment (Goldberger, 1997; Kobayashi & Musha, 1982; Lipsitz & Goldberger, 1992; Peng et al., 1993).

The complexity of the cardiovascular system is reflected in the linear and non-linear influences in its environment. Many of the same extrinsic and intrinsic factors that influence the ANS also influence HRV. Extrinsic factors include posture (Montano et al., 1994), exercise (Pober, Braun, & Freedson, 2004; Uusitalo, Laitinen, Vaisanen,
Intrinsic factors include respiratory sinus arrhythmia (Yasuma & Hayano, 2004), sleep processes (Viola et al., 2002), temperature and neuroendocrine regulation (Task Force, 1996), and circadian rhythms (Bonnemeier et al., 2003; Molgaard, Sorensen, & Bjerregaard, 1991; Scheer, Van Doornen, & Buijs, 2004).

Gender-related differences in HRV have not been widely investigated and observations appear dependent on several factors, including age (Bonnemeier et al., 2003; Jensen-Urstad et al., 1997). Ageing has been observed to alter HRV extensively, as autonomic regulation becomes attenuated and hormononal and thermoregulatory rhythms lose strength (Vigo, Guinjoan, Scaramal, Siri, & Cardinali, 2005). Furthermore, these effects are compounded by the generalised effects of diminished arterial compliance, blunted baroreceptor sensitivity, and aging of cardiac pacemaker cells. The loss of regulatory components associated with aging leads to a loss of complexity. The loss of complexity, manifest as restricted or reduced HRV, impairs the ability to adapt to physiological challenges and promotes greater cardiovascular risk (Iyengar, Peng, Morin, Goldberger, & Lipsitz, 1996; Korkushko, Shatilo, Plachinda Yu, & Shatilo, 1991; Yamanaka & Honma, 2006).

Chemical agents that modulate the ANS also influence HRV, including certain medicines, caffeine, tobacco, and alcohol (Kupari, Virolainen, Koskinen, & Tikkanen, 1993; Task Force, 1996). Smoking (Hayano et al., 1990) and alcohol consumption (Kupari & Koskinen, 1993) are related to impaired autonomic function, which can affect HRV. However, lifestyle factors such as these also show gender (Jensen-Urstad, Jensen-Urstad, Ericson, & Johansson, 1998) and personality (Kamada, Miyake, Kumashiro, Monou, & Inoue, 1992) variation.

Pathologies such as chronic renal failure (Cloarec-Blanchard, Girard, Houhou, Grunfeld, & Elghozi, 1992), diabetes (Ewing, Borsey, Bellavere, & Clarke, 1981), and neurological disorders (Korpelainen, Sotaniemi, Huikuri, & Myllya, 1996) can also influence HRV. The hypothalamic-pituitary-adrenal (HPA) axis is generally regarded as a complex neuroendocrine system, which influences and regulates processes such as digestion, immunity, stress reactions, and mood. Dysregulation of
the HPA axis has been associated with alcoholism (Adinoff, Iranmanesh, Veldhuis, & Fisher, 1998; Thayer, Hall, Sollers, & Fischer, 2006), and impairment of the HPA axis in heavy drinkers appears to lower HRV (Thayer et al., 2006). Dysregulation of the HPA axis is also linked with epilepsy (Zobel et al., 2004) and disorders such as depression, schizophrenia, and anxiety (Drevets, 1999; Thayer & Friedman, 2002). An impaired HPA axis from affective disorders such as these can also alter cardiac autonomic tone (Bar et al., 2004; Cohen & Benjamin, 2006; Udupa et al., 2007).

An increase in body weight, by as little as 10%, can influence HRV by reducing parasympathetic function (Hirsch, Leibel, Mackintosh, & Aguirre, 1991; Hirsch, Mackintosh, & Leibel, 1991). The same investigators suggest that the reduction in parasympathetic function may be one of the mechanisms underlying cardiac alterations (such as arrhythmias) that are often observed in obesity. Elevated insulin associated with obesity and diabetes is widely recognised as a risk factor for cardiovascular disease. Yet, evidence regarding the role of elevated insulin in changes to cardiac autonomic function is conflicting; for example, high insulin levels seem to trigger sympathetic activation (Tack et al., 1998; Vollenweider et al., 1993), but increased sympathetic activity has been shown to directly induce insulin resistance (Flanagan et al., 1999).

**Measurement and parameters of heart rate variability**

Quantifying cardiac autonomic activity can be done using time and frequency domains or non-linear methods (Stein & Kleiger, 1999). One of the most widely used frequency domain analyses is power spectral density (PSD). Power spectral density is a non-invasive linear method that extracts periodic oscillations from HRV data. The oscillations are then expressed as a function of frequency and the amplitude quantified by assessing the area (or PSD) under the curve within each frequency band (Sztajzel, 2004; Zhong, Jan, Ju, & Chon, 2006).

The popularity of PSD stems from the computational ease provided by the non-parametric Fast Fourier Transform. Auto-regressive parametric methods are also used for PSD but tend to be more complex and the suitability of the chosen model requires
verification (Sztajzel, 2004; Zhong et al., 2006). The power spectrum of HRV is routinely classified into four frequency bands (Task Force, 1996):

1. ultra-low frequency (ULF) (0.0033-0.05 Hz)
2. very low frequency (VLF) (0.003-0.04 Hz)
3. low frequency (LF) (0.04-0.15 Hz)
4. high frequency (HF) (0.15-0.4 Hz).

The boundaries between frequencies are not fixed and may vary with changes to the autonomic control of the heart. Therefore the ranges defined represent a pragmatic delineation rather than a functional basis (Sztajzel, 2004; Task Force, 1996).

Each frequency band is associated with a variety of influences on HRV. In order to extract the signal, the low oscillation of ultra-low frequency requires longer-term HRV recordings (>20 minutes). The circadian rhythm is the most prominent oscillation in the ULF band, a major component of which is the diurnal fluctuation of ANS activity (Buijs, van Eden, Goncharuk, & Kalsbeek, 2003; Stauss, 2003; Sztajzel, 2004). The ANS circadian rhythm is generated by fluctuations within the hypothalamus, which also influences ULF via other circadian rhythms such as metabolic and behavioural (Bartness, Song, & Demas, 2001; Stauss, 2003).

Thermoregulation has been suggested as the primary influence on VLF, although diverse stimuli and conditions have also been found to contribute. These include haemorrhage, acidosis, alkalosis, high altitude, posture, congestive heart failure, breathing patterns, and spinal reflexes (Stauss, 2003). While the determinants of the VLF and ULF bands is not as well known as the other bands, these components are thought to account for as much as 95% of 24-hour total power (Pikkujamsa, 1999).

The remaining frequency bands can be extracted with shorter-term HRV recordings. The low frequency band has previously been proposed as an index of pure sympathetic activity. Recent studies suggest that LF is mediated by both sympathetic and parasympathetic traffic (Stauss, 2003; Sztajzel, 2004; Task Force, 1996; Zhong et al., 2006). Baroreceptor activity is thought by some to be the predominant influence on LF (Sleight et al., 1995), although some human (Cooley et al., 1998) and animal data (Montano et al., 1996) suggests a medullary origin. Since LF is not specific for
either ANS division it is usually not considered a general index of sympathetic cardiac control or sympathetic-vagal balance (Task Force, 1996).

By comparison, the high frequency band is dominated by parasympathetic (vagal) activity (Stauss, 2003; Task Force, 1996). As vagal governance of respiration mediates HF, the HF band is also described as the ‘respiratory frequency’. Respiratory-driven vagal modulation of heart rate is termed respiratory sinus arrhythmia (RSA), which represents spontaneous fluctuations resulting from central coupling between respiratory oscillators in the brainstem and autonomic outflow (Stauss, 2003; Szajzel, 2004). During inspiration this coupling manifests as inhibited vagal activity, causing the balance of ANS cardiac activity to tip in favour of the sympathetic system and thus increase heart rate. Correspondingly, expiration releases the inhibition of vagal activity and heart rate decreases (Stauss, 2003; Szajzel, 2004). The frequency and amplitude of the respiratory-driven cardiac fluctuation is also dependant on respiratory rate and depth.

To summarise, research literature clearly indicates that parasympathetic activity is the greatest determinant of all HRV (Akselrod et al., 1981; Pagani et al., 1991; Task Force, 1996; Taylor, Carr, Myers, & Eckberg, 1998). However, intrinsic rhythms such as thermoregulation and extrinsic factors such as posture must be included as part determinants of HRV.

Limitations of heart rate variability as an indicator of ANS activity

Heart rate variability data is not a direct measure of cardiac autonomic activity. Consequently, a potential exists for incorrect conclusions and unfounded extrapolations when interpreting HRV data and deriving inferences regarding the state and function of the ANS based on such data (Berntson et al., 1997; Mookherjee, 2003; Niskanen et al., 2004). Furthermore, analysis of HRV is a rapidly evolving field and there is disagreement regarding quantitative approaches to HRV measurement due to the multiple and often overlapping non-linear influences (Belova, Mihaylov, & Piryova, 2007; M. Brennan, Palaniswami, & Kamen, 2001; Guzzetti et
al., 2005; Malpas, 2002; Radespiel-Troger, Rauh, Mahlke, Gottschalk, & Muck-Weymann, 2003).

The Task Force (1996) points out that measurement, analysis, and interpretation of HRV has clear pitfalls and caveats that need to be considered by researchers and clinicians utilising these methods. Research literature is equivocal regarding the most accurate HRV parameter for clinical use, underpinned by the need for clear elucidation of the putative link between reduced HRV and mortality (Sztajzel, 2004). Large intra-individual variation in autonomic function, such as age, gender, pharmacological agents, psychology, and concomitant disease are also recognised as factors that prevent prescription of a standardised method of HRV assessment (Abdel-Rahman, Merrill, & Wooles, 1994; Huikuri et al., 1990; Kupari, Virolainen, Koskinen, & Tikkanen, 1993).

A further limitation is that stability over time of short-term HRV recordings has shown variable reliability (Kleiger et al., 1991; Sandercock, Bromley, & Brodie, 2005; Tarkiainen et al., 2005). Coefficients of variation have ranged from <1% to >100%, and with the absence of Task Force recommendations, targets for acceptable levels of reliability (4-30%) have been adopted by various investigators on an apparently arbitrary basis. Despite the wide range of reliability described in the literature, satisfactory intra-individual reproducibility of 24-hour recordings has been demonstrated in both healthy populations and those with known heart disease (Huikuri et al., 1990; Kleiger et al., 1991). With the issues surrounding the stability of HRV data, the Task Force has recommended that, for clinical purposes, HRV should only be used to warn of early diabetic neuropathy and predict risk of death after acute myocardial infarction (AMI).

Heart rate variability has also shown modest or limited sensitivity, specificity, and positive predictive accuracy in cardiovascular disease. The Task Force has advised that predictive relationships based on HRV can be degraded by cardiac or neural abnormalities, autonomic interactions, and respiratory parameter variability. Moreover, artifact and ectopic beats during HRV recording can considerably interfere with estimates and may invalidate the measurement or lead to over-editing of raw data (Pikkujamsa, 1999). The differences in diagnostic odds of HRV between recent and
previous literature may reflect changes to early treatment protocols of post-AMI patients. Or, in high-risk patients, the evidence could suggest that by itself HRV is insufficient as a risk stratifier (Stein & Kleiger, 1999; Sztajzel, 2004). Consequently, the limitations of HRV have prompted some authors to reiterate that HRV only reflects fluctuation in cardiac autonomic input and does not represent mean autonomic tone (Malik & Camm, 1993; Parati, Mancia, Di Rienzo, & Castiglioni, 2006).

The limitation of multiple and overlapping influences on HRV can complicate controlling for extraneous variables in experiments. In some studies employing HRV to evaluate the autonomic effects of manual therapy techniques, results may have been confounded by other influences; for example, vestibular stimulation (Budgell & Hirano, 2001) or differences in the application of the technique between practitioners (Zhang et al., 2006). In another investigation, Cooper (2006) applied two innocuous non-spinal techniques and two innocuous spinal techniques to subjects across a single 80 minute data collection period. Cooper also “failed to show statistically significant pre-post intervention changes” from application of the four consecutive techniques. The author suggested that the lack of significant variation in results was partly influenced by skin stimulation, habituation to subsequent stimuli, and psychological and physiological variation. Recently, Grimm, Cunningham, and Burke (2005) evaluated differences in autonomic function between healthy and injured subjects. Although Grimm observed significant differences in skin conductance and baroreceptor sensitivity, no difference was found in the low and high frequency R-R interval variables of the injured subjects. These results may have been due to variables associated with heterogeneity of tissue damage between subjects, and natural intra-individual variation in ANS indices from hormonal or genetic differences.
Pain

Introduction

Pain has been defined as “An unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage” (Merskey & Bogduk, 1994, p.210). One component of pain, nocioception, which describes the physiological signal that occurs from stimulation of free nerve endings (nociceptors) by stimuli considered capable of causing tissue damage (Siddall & Cousins, 1997). But, as Melzack and Wall (1965) state, the experience of pain and nocioception are not one and the same. Pain is considered to involve higher centres associated with cognition, emotion, and sensation, and that actual or potential tissue damage stimulates feedback circuits that result in behavioural and autonomic responses. Although clinical pain management can be hobbled by the mind-body dichotomy, greater knowledge of the affective-emotional dimension of pain has lead to better understanding of chronic pain, coping strategies, and effective interventions.

Physiological or sensory dimension of pain

The physiology of pain involves activation of nociceptors and the relay of stimuli to the brain where it is recognised as potentially harmful (Siddall & Cousins, 1997). More commonly, however, insults to the body injure a variety of tissues and produce inflammation. Tissue damage and inflammation cause a cascade of events to occur such as lowered threshold of sensory neuron activation, increased rate of firing, and increased responsiveness to thermal stimuli (Siddall & Cousins, 1997). Concurrently, inflammatory mediators cause silent sensory fibres, which are usually non-responsive under normal conditions, to react to mechanical pressure (Siddall & Cousins, 1997). The term given to the outcome of this process is peripheral sensitisation. Peripheral sensitisation allows a low-intensity stimulus within the injured area to be perceived as painful (Butler, 2000; Siddall & Cousins, 1997). The clinical correlate of peripheral sensitisation is primary hyperalgesia, which is heightened responsiveness to a painful stimulus within the injured area (Graven-Nielsen & Mense, 2001). If nocioception is

---

5 Components of pain: nocioception (stimulation of free nerve endings from noxious or tissue damaging stimulus), pain perception (i.e. cognitive or sensory awareness of pain), pain behaviours such as wincing that are observable behavioural responses, and personal suffering which is the emotional reaction to pain (Loeser & Melzack, 1999; Merskey & Bogduk, 1994).
prolonged, gene induction in the dorsal root ganglia and the dorsal horn of the spinal cord occurs. This may result in central sensitisation, a term that describes longer-term alteration in sensory nerve responsiveness, the development of cellular ‘memory’ for pain, and the development of connections with nerves that conduct mechanical stimuli (Siddall & Cousins, 1997). Central sensitisation causes heightened sensitivity of sensory neurons in a wider area by contributing to the phenomena of allodynia\(^6\) and secondary hyperalgesia\(^7\) (Butler, 2000; Siddall & Cousins, 1997).

**Affective or emotional dimension of pain**

Many factors are believed to account for the affective-emotional dimension of pain. Subject gender (Keogh, Hatton, & Ellery, 2000), experimenter gender (Aslaksen, Myrbakk, Hoifodt, & Flaten, 2007; Levine & De Simone, 1991), negative affect (Rhudy & Meagher, 2003), and fear (Bradley, Silakowski, & Lang, 2008) all modulate evaluation of pain and subsequent coping strategies.

Attentional bias is a term used in psychology and pain literature which refers to the tendency toward a particular cognitive strategy to cope with pain. Attentional biases include ignoring, distraction, catastrophising, positive appraisal, reinterpretation, and praying (Boothby, Thorn, Stroud, & Jensen, 1999, pp. 343-359.; Keogh et al., 2000). Research demonstrates that attentional biases such as distraction, avoidance, or focused attention, affect the speed and accuracy of noxious stimulus detection and therefore the perception of pain (Miron, Duncan, & Bushnell, 1989).

Attentional bias also influences the perceived intensity or unpleasantness of the stimulus (Keogh et al., 2000; Miron et al., 1989). Furthermore, a bias of preferential attention towards pain is proposed to be an important factor in the development of vulnerability, which can predispose healthy individuals to react negatively to pain (Keogh, Ellery, Hunt, & Hannent, 2001). An important component of attentional bias is fear of pain. Fear of pain and hypervigilance are thought to predispose individuals to chronicity, distress, disability, and greater use of healthcare resources (Keogh et al.,

---

\(^6\) Allodynia: Pain due to a stimulus which does not normally provoke pain (Merskey & Bogduk, 1994, p. 210)

\(^7\) Hyperalgesia: An increased response to a stimulus which is normally painful (Merskey & Bogduk, 1994, p. 211). Secondary hyperalgesia is heightened responsiveness of the surrounding uninjured tissue (Butler, 2000).
In chronic musculoskeletal pain this postulate is known as the ‘fear-avoidance model’ (Asmundson & Hadjistavropoulos, 2007) or ‘fear of (re)injury model’ (Vlaeyen & Linton, 2000).

Threat value is an important factor in pain as perceived threat demands attention and higher centre resources (Crombez, Baeyens, Vansteenwegen, & Eelen, 1997; Moseley, Brhyn, Ilowiecki, Solstad, & Hodges, 2003). Threat value is highly subjective, and intrinsically linked to fear of pain, attentional bias, control, and context (Moseley et al., 2003; Munafo & Stevenson, 2003; Vlaeyen & Linton, 2000). The situational context and meaning ascribed to pain has been shown to have a particularly significant influence on threat value (Bayer, Baer, & Early, 1991; Smith, Gracely, & Safer, 1998). To illustrate, Moseley (2003) uses the example that a professional violinist with a minor finger injury will experience greater pain as the damage is overlaid with a threat to livelihood and identity. In a person whose livelihood is not dependant on a high level of finger function, the same minor finger injury may be experienced as less threatening, and therefore less painful.

A further element associated with threat is that of predictability. Rhudy & Meagher (2000) have proposed that the possibility of shock induces anxiety, whereas predictable yet unavoidable shock induces fear. These two emotions have differing effects on pain. Fear appears to evoke a phenomenon known as ‘stress-induced analgesia’ where pain is attenuated by release of endogenous opioids (Rhudy & Meagher, 2000). Anxiety, a future-oriented emotion, results in apprehensive anticipation of potential threat which leads to increased environmental and somatic (body) scanning. The anxiety state can therefore facilitate increased sensory receptivity and enhance pain (Rhudy & Meagher, 2000). Furthermore, the anticipation of threat in the absence of actual exposure is enough to potentiate startle reflexes and autonomic responses (Bradley et al., 2008).

The autonomic nervous system and pain

In broad terms, the ANS response to noxious, tissue-damaging events may be classified as being specific or generalised. Reactions to pain and injury are widely
recognised to include heart rate changes, sweating, and raised serum and salivary cortisol levels. When confronted with actual or potential damage, the ANS activates higher centres to co-ordinate strategies and patterns of defence behaviour (Butler, 2000). In a review on pain and autonomic reactions, Benarroch (2001) outlines that these higher centre mechanisms are invoked via direct projections from the dorsal horn of the spinal cord to the brainstem, and provide the basis of autonomic, endocrine, and behavioural responses. The responses include anti-nociceptive mechanisms such as cardio-respiratory and renal system changes, analgesia which is independent of endogenous opioids, and motivational modulation of pain (Benarroch, 2001).

In the periphery, sympathetic neurotransmitters can maintain and enhance nociocception by augmenting chemical mediators and growth factors (Butler, 2000). Also observed to occur in the periphery is coupling between sensory and sympathetic fibres. This coupling has been observed in the sympathetic chain ganglia, and between sensory nerves and sympathetic nerves traversing or synapsing within the dorsal root ganglia before entering the spinal cord. The complex interaction between sensory and sympathetic fibres has been shown to modulate activity in the dorsal root ganglia (Siddall & Cousins, 1997). This interaction has prompted some to postulate that changes associated with a phenomenon such as central sensitisation may mean that activity in sympathetic fibres could lead to abnormal responsiveness of sensory fibres (Koltzenburg & McMahon, 1991).

Despite the postulated sympathetic contribution to pain, there is evidence to challenge sympathetic involvement. Schott (1994; 1999) summarises research reporting a lack of correlation between clinical features of pain and known sympathetic effects, such as skin temperature and moisture changes. Furthermore, while direct damage to the sympathetic nervous system is believed to cause pain, it appears that pathological lesions and stimulation of sympathetic nerves only occasionally cause pain (Schott, 1999). Pain associated with lesions involving sympathetic fibres, such as tumours, vascular disease, and trauma, has therefore been speculated to be associated with visceral sensory nerves travelling with the sympathetic nerves, and not the sympathetic nerves themselves (Schott, 1999).
**Delayed onset muscle soreness**

Delayed onset muscle soreness (DOMS) is the sensation of dull aching pain and discomfort felt after unaccustomed or strenuous exercise (Croisier et al., 2003; Friden & Lieber, 2001). The soreness peaks approximately 24-48 hours following exercise, is accompanied by stiffness, weakness, and tenderness, and disappears completely within 3-7 days of the precipitating activity (Armstrong, 1984; Graven-Nielsen & Arendt-Nielsen, 2003). Delayed onset muscle soreness is distinguished from temporary soreness, which is moderate pain felt during the final stages of fatiguing exercise and believed to be caused by metabolic waste (Croisier et al., 2003).

Clinical studies have found that the most effective way to induce DOMS is with eccentric exercise. During eccentric exercise the contracting muscle is forcibly lengthened, placing high tension on muscle fibres and connective tissue (Proske & Morgan, 2001). The disruption to skeletal muscle from unaccustomed exercise is not considered permanent and is not associated with disruption to articular, vascular, neurological, or osseous structures (Friden, 2002; Proske & Morgan, 2001).

**Physiology of delayed onset muscle soreness**

The mechanisms underlying DOMS are not completely understood. Earlier theories included spastic contracture of the muscle (De Vries, 1966), structural damage to the muscle fibres and connective tissue (Armstrong, 1984), and a loss of calcium homeostasis in the injured fibre (Armstrong, 1990). The most recent and best supported hypothesis is mechanical stress triggering structural damage and a subsequent inflammatory response (Lieber & Friden, 2002).

Described in temporal terms, unaccustomed or strenuous exercise causes micro-damage to muscle fibres but no soreness immediately after exercise. The mechanical disruption disturbs the chemical milieu of muscle which is proposed to trigger a subsequent inflammatory response. The inflammatory response develops over several hours, and inflammatory mediators such as bradykinin are believed to stimulate free nerve endings and sensitise them so as to respond to normal innocuous stimuli (also known as peripheral sensitisation) (Mense & Meyer, 1988; Proske & Morgan, 2001).
On microscopy of muscle tissue taken at biopsy, there is direct evidence of muscle damage such as disruption to muscle fibres and regional disorganisation of filaments (Proske & Morgan, 2001; Yu, Carlsson, & Thornell, 2004). Structural damage is also reflected in objective tests of the mechanical properties of muscle: DOMS is associated with decreased active tension, increased passive tension, altered muscle length (shorter), and reduced range of motion (Friden & Lieber, 2001). Changes in serum and urine constituents also indicate increased permeability or breakdown of the muscle cell membrane, increased cell disruption, and collagen and myosin turnover (Blais, Adam, Massicotte, & Peronnet, 1999).

There is some debate within the literature regarding the proposed mechanisms involved in DOMS. Inflammation subsequent to damage is not always confirmed (Malm et al., 2000; Nosaka, Newton, & Sacco, 2002; Vincent & Vincent, 1997) and the muscle damage theory has too been challenged (Friden & Lieber, 2001; Newham, 1988). Blaise et al. (1999) contend that while some inflammatory mediators are known to stimulate nociceptors, the involvement of many of these substances in DOMS remains largely hypothetical.

Conjecture also surrounds the involvement of connective tissue in the DOMS process. Graven-Nielsen, Gibson, and Arendt-Nielsen (2006) have recently implicated the role of fascial tissue in DOMS. Based on subjective reports by research participants, other investigators have proposed that mechanical damage to the musculotendinous junction (MTJ) stimulates the dense free nerve endings found at the MTJ and signals nociception (Cleak & Eston, 1992). Yet, the nociceptive characteristics of tendon are not clear (Gibson, Arendt-Nielsen, & Graven-Nielsen, 2006) and other studies indicate this effect may depend on the amount of damage at the MTJ (Bajaj, Graven-Nielsen, & Arendt-Nielsen, 2001; Eston, Critchley, & Baltzopoulos, 1994), and how large the muscle attachment area is (Bajaj et al., 2001; Brand, Beach, & Thompson, 1981).

Some reports propose that central mechanisms may be important in pain associated with DOMS. Gibson et al. (2006) suggest that central sensitisation may be involved after finding greater referred pain frequency and enlarged pain areas during DOMS.
compared to pre-DOMS. Similarly, Bajaj et al. (2001), found correlations between tenderness at the musculotendinous junction and the sensory and affective components of the pain-rating index. These findings were backed by the simultaneous presence of a significant reduction in mean arterial pressure during peak DOMS, suggesting central mechanisms were involved. Several authors claim that input from mechanically sensitive muscle spindles and golgi tendon organs interact with neurons in the spinal cord that also have connections to pain pathways (Barlas, Walsh, Baxter, & Allen, 2000; Weerakkody, Whitehead, Canny, Gregory, & Prosko, 2001). Another related suggestion is that inhibitory neurons in the spinal cord become excited from DOMS/injury and interact with fibres relaying noxious stimuli.

The theories regarding the involvement of central mechanisms in DOMS parallel those of autonomic reflexes, as muscle sensory fibres have elicited cardiovascular changes via spinal cord interactions (Coote & Bothams, 2001; Kerman & Yates, 1999; Sato, Sato, & Schmidt, 1979). Recently, Gladwell et al. (2005) have observed that muscle fibres responding to mechanical deformation, specifically stretch, selectively inhibited cardiac vagal tone to produce tachycardia. Therefore muscle mechanoreceptors could conceivably play a part in the reflex connections that exist to mediate somato-autonomic activities and in fight or flight responses.

Despite the exact mechanisms being unclear, there is general agreement that DOMS appears to be beneficial in terms of the objective properties of muscle. Yu et al. (2004) propose that remodelling as opposed to damage is the mechanism at work, supported by the so-called ‘repeated-bout effect’. The repeated-bout effect occurs when subsequent bouts of eccentric exercise, from 1-10 weeks after the initial bout, produce much less muscle damage – implying an adaptive and protective function (Ebbeling & Clarkson, 1989; Golden & Dudley, 1992; Nosaka, Clarkson, McGuiggin, & Byrne, 1991; Paddon-Jones, Muthalib, & Jenkins, 2000). Even second bouts performed during the early recovery period (1-5 days) of an initial bout have not exacerbated muscle damage or retarded the recovery process (Ebbeling & Clarkson, 1989; Paddon-Jones et al., 2000), with some studies showing this effect regardless of the intensity and volume of eccentric action performed in the second bout (Chen & Nosaka, 2006). Light eccentric exercise, which does not significantly change markers
of muscle damage, is also believed to confer a protective effect (Lavender & Nosaka, 2008).

A combination of neural, cellular and mechanical factors is thought to be responsible for the repeated bout effect (McHugh, 2003). Connective tissue shows greater resilience to subsequent provocation, repair mechanisms of contractile tissue show altered fibre-type composition and protein gene-expression, and motor unit recruitment patterns better distribute workload (Croisier et al., 2003; Mair et al., 1995; Nosaka & Clarkson, 1995). The suggestion of adaptation supports the idea that mild eccentric exercise may have a useful clinical application in prevention of major injury (Croisier et al., 2003; Friden, 2002; Proske & Morgan, 2001), although it is not currently known how much muscle damage might be necessary for producing such an adaptive effect (Lavender & Nosaka, 2008).

**Delayed onset muscle soreness as a model of pain**

Pain is a complex phenomenon involving peripheral mechanisms and higher centre involvement (Melzack & Wall, 1965). Delayed onset muscle soreness as a model of pain in terms of the physiological-sensory dimension, involves micro-damage to tissue and stimulation of free nerve endings from the breakdown products of tissue damage. Furthermore, the proposed mechanisms underlying DOMS also include inflammatory response to such damage. The inflammatory substances implicated, such as bradykinin, are known to stimulate and sensitise free-nerve endings that relay information regarding noxious stimuli. As a model of pain in terms of the affective or emotional dimension of pain, DOMS would, like other noxious stimuli, be dependant upon aspects such as cognitive appraisal of threat (Jackson et al., 2005; Moseley et al., 2003), environmental context (Malenbaum, Keefe, Williams, Ulrich, & Somers, 2008), coping strategies (Crombez, Eccleston, De Vlieger, Van Damme, & De Clercq, 2008), and prevailing emotions (e.g. fear or anxiety) (Rhudy & Meagher, 2000).

As the aim of this project was to explore mild to moderate musculoskeletal injury on the ANS, DOMS was selected as a model of tissue injury that could be induced under controlled conditions. Compared to naturally occurring injuries, using a DOMS-
model would partially control for variability in severity and damage to tissues other than myofascia. The temporal nature of DOMS was considered useful to compare subjective appraisal of pain with the sequence of events thought to occur in DOMS. Since DOMS is not associated with chronicity, and believed to have an adaptive role, DOMS may be considered an ethically appropriate model of pain to use in experimental research.
Summary and aims of research

Osteopaths have historically maintained a central tenet within osteopathic literature, namely that visceral and musculoskeletal disturbance can affect ANS balance. The perturbation of the ANS from such disturbance is said to affect structures elsewhere in the body via common autonomic segments. This concept provides part of the rationale for treatment approach and therapeutic techniques such as spinal manipulation. Yet, while ANS changes have been shown to occur in response to noxious stimuli and with some diseases, studies to date do not support the osteopathic concept that non-pathological disturbance can perturb the ANS. Furthermore, claims that osteopathic techniques such as spinal manipulation can address purported ANS perturbation have not been validated.

The mechanisms underlying spinal manipulation have not been fully elucidated. There is also limited evidence regarding the other links in the purported causal chain between visceral and musculoskeletal disturbance and the ANS. Nonetheless, the primary focus of previous investigations has been the cause-effect relationship between spinal manipulation and the ANS. Osteopathy has a number of areas to evaluate, for example: whether mild to moderate musculoskeletal disturbance affects the ANS; what magnitude of effect spinal manipulation has on the ANS; whether the direction of such an effect is favourable or not; and the clinical significance of the techniques. The objective of the investigation documented in Section 2 of this dissertation was to determine whether a mild musculoskeletal disturbance, such as that evident in DOMS, has the capability to influence the ANS as measured by heart rate variability.
References


velocity low-amplitude thrust technique on the cutaneous blood flow in the lower limb. 


Section 2: Manuscript

Note
This manuscript has been prepared in accordance with the Guide for Authors for the International Journal of Osteopathic Medicine (See Appendix E).
The effect of pain associated with delayed onset muscle soreness on the autonomic nervous system as measured by heart rate variability
The effect of pain associated with delayed onset muscle soreness on the autonomic nervous system as measured by heart rate variability

Larissa Morgan

School of Health Science
Unitec New Zealand
Private Bag 92025, Auckland
New Zealand

Tel: +64 9 815 4321 x8642
Fax: +64 9 815 4573
Email: larissam@woosh.co.nz
Abstract

Background: A central concept within osteopathy is that disturbance of musculoskeletal or visceral structures can perturb the autonomic nervous system (ANS). Such perturbation is postulated to generate reflexes that can affect other structures with the same autonomic innervation. Within osteopathy, the effects of this purported process are considered amenable to manual therapy techniques, such as spinal manipulation. However, scientific research investigating this process is limited and often inconclusive, and despite evidence demonstrating that spinal manipulation affects the ANS, the clinical relevance of such an effect has had limited discussion.

Objective: The objective of this study was to examine the effect of a mild to moderate musculoskeletal ‘disturbance’, such as delayed onset muscle soreness (DOMS), on the ANS as measured by heart rate variability (HRV), in healthy young males.

Methods: A time series single-subject design was conducted on six subjects (age range 23 to 36 years). Heart rate was recorded daily for 15 minutes over five consecutive days to establish baseline (Phase A) and then daily for nine consecutive days after an exercise protocol to induce DOMS (Phase B). Pain intensity scores were recorded twice daily throughout Phase B. Frequency spectra for HRV data was extracted and five minutes of normalised R-R intervals were plotted with pain intensity data against time. Visual analysis of changes to level, trend, and stability of HRV and pain intensity data was then performed.

Results: Within the confines of this study, the results indicated that changes to HRV did not occur as a result of pain associated with DOMS. Although slight differences in the stability of HRV data and slope of trend line occurred in some subjects, no consistent pattern emerged that was attributable to DOMS.

Conclusion: Delayed onset muscle soreness was not clearly associated with changes to the autonomic output to the heart, as measured by HRV, in healthy males.

Key terms: Autonomic nervous system; Heart rate variability; Delayed onset muscle soreness; time-series design.


**Introduction**

A central concept within osteopathic texts is that disturbance of musculoskeletal structures can perturb the autonomic nervous system (ANS). Perturbation of the ANS is believed to potentially influence the function of viscera or other musculoskeletal structures supplied by common autonomic segments. The osteopathic concept that disturbance to visceral and musculoskeletal structures can have effects elsewhere in the body via the ANS, is commonly described in historical and anecdotal reports but has not been adequately investigated. This concept also provides part of the rationale for the osteopathic treatment approach and the use of spinal manipulation techniques. Although research indicates that noxious and long-term musculoskeletal stimuli can produce changes in the ANS, the effects from mild to moderate musculoskeletal disturbance are less apparent.

Osteopathic literature has begun to gather evidence on one aspect of the concept that musculoskeletal or visceral disturbance can perturb the ANS: namely, whether manual therapy techniques, such as spinal manipulation, influence autonomic activity. Recent evidence has demonstrated that spinal manipulation appears to exert an effect on the ANS, however, these studies do not indicate the magnitude of effect nor whether the effect on the ANS is favourable or unfavourable. Furthermore, it is not clear how clinically beneficial techniques that putatively influence the ANS are, nor how necessary such techniques are to patient care.

Recently, a case series study by Grimm et al. investigated soft tissue injury and ANS indices. Although Grimm reported alterations in cardiac and peripheral autonomic activity amongst injured subjects, there is scant literature investigating a possible relationship between soft tissue injury and ANS perturbation. With few studies, and no clear physiological mechanisms, evidence to support or refute the osteopathic concept that musculoskeletal disturbance can perturb the ANS is lacking. Furthermore, it is unclear whether it necessarily follows that such ANS perturbation will have an unfavourable effect. If osteopathic techniques can modulate ANS activity, do they evoke an ANS response beyond any localised or short-term general effect, to ameliorate the so-claimed effects further afield? Establishing the nature and
significance of any purported links is important to not only evaluate tenets within osteopathy, but may be of clinical relevance to those with acute injury or those in chronic pain states.

Delayed onset muscle soreness (DOMS) is temporary soreness commonly experienced after unaccustomed or strenuous exercise. An initial step in establishing the nature and significance of the purported links between the ANS and musculoskeletal structures, is to undertake preliminary work to establish 1) if DOMS is a viable model for investigating this ANS-injury relationship, and 2) if there is an association between DOMS and ANS disturbance that is consistent with the osteopathic concept. The aim of this preliminary study was to determine whether the physiological effects of DOMS, as a model of mild to moderate musculoskeletal pain, would influence the ANS as measured by heart rate variability (HRV).
Methods

Design

A time-series A-B experimental design was used for this preliminary study. Time-series or single-subject designs are a useful low-cost approach to provide preliminary information about possible causal relationships.13-15 Such designs are logistically simple and may provide avenues for further investigation with more robust group designs such as randomised, controlled, experimental studies.

Subjects

Subjects were recruited via word of mouth and poster advertising at Unitec New Zealand. All subjects satisfied exclusion and inclusion criteria. Exclusion criteria were: disorders of the immune, endocrine, and musculoskeletal systems; recent or unresolved trauma; psychological disorders; and medication known to influence the ANS or cardiovascular system. Exclusion criteria also included participation in high-intensity or frequent cardiovascular or resistance exercise (five or more days per week), such as high-intensity cycling, running, or weight training. Subjects were required to be non-smokers, with a body mass index (BMI) between 18.5 and 30, and not experiencing pain or discomfort at the time of recruitment. The Unitec Research Ethics Committee approved the study and written informed consent was obtained from all subjects [see Appendix A].
Variables and Operational Definitions

Independent variable

The intended tissues in which DOMS was to be induced were the elbow flexor, and possibly forearm flexor, muscles of the non-dominant arm. The manner in which DOMS was induced was consistent with previous studies on DOMS, utilising eccentric contraction of the elbow and forearm flexor muscles to produce controlled elbow extension to the end of range. The maximum weight able to be performed for a single repetition of elbow flexion, or one repetition maximum (1RM), was ascertained, and isokinetic eccentric contraction of the elbow and forearm flexors was performed using a dumbbell weight calculated to be 40-50% of the 1RM. Completion of the exercise protocol was determined to be failure to complete an eccentric contraction, that is, inability to control the decent of the dumbbell.

Dependant variable

A wireless heart rate monitor (Polar S810i, Polar Electro Oy, Finland) was used to collect consecutive beat-to-beat electrocardiogram signal, which was later analysed in HRV Analysis Software v1.1 (The Biomedical Signal Analysis Group, Kuopio, Finland). The Polar S810i has been validated and good agreement with other electrocardiogram instruments has been demonstrated. All data was stored on a personal computer for off-line data analysis.

The Short Form McGill Pain Questionnaire (SF-MPQ) was used to measure sensory and affective qualities of pain and perceived pain intensity. A standardised 100mm horizontal visual analogue scale (VAS) contained in the SF-MPQ, anchored on the left with “no pain” and the right with “worst possible pain”, was also used to measure pain intensity. The SF-MPQ and VAS are widely used in research and are generally considered to be valid and reliable instruments for pain assessment.
Procedure

The study was divided into two phases as illustrated in Figure 1. Phase A consisted of heart rate recording for 15 minutes per day, between the hours of 1600 and 2000, over five consecutive days. Phase B involved an eccentric exercise protocol on day six that was intended to induce DOMS, and continued daily heart rate recording and completion of the SF-MPQ approximately every 12 hours until the end of data collection on day 14.

Each subject received a project information sheet [see Appendix B], operational instructions for the heart rate monitor [see Appendix C], and a DOMS information sheet [see Appendix D]. Phase B of the data collection period was outlined to subjects including the exercise protocol. Correct use of the heart rate monitor was demonstrated, and subjects practiced placement of equipment and recording of heart rate in a seated position until deemed by the investigator to be competent and confident with operation. Each subject was instructed on the daily protocol of data collection, and requested to refrain from exercise and from consumption of stimulants, alcohol, and food three hours prior to recording sessions. Subjects were supplied a nature documentary DVD (The Living Planet)\textsuperscript{25} to watch during heart rate recording. Use of the documentary was intended to provide similar recording conditions between measurement sessions as data collection was undertaken in the subject’s home. The documentary was pre-screened by the investigator and did not

![Figure 1](image-url)
contain any content that would potentially startle subjects or evoke strong emotional responses.

**Intervention**

Subjects performed the exercise protocol during the morning of day six. Pain intensity scores were recorded prior to the exercise. The 1RM for the non-dominant arm was determined, and a dumbbell of approximately 40-50% of the 1RM selected. Subjects were seated at one end of a standard gym training bench and instructed on positioning to provide full elbow extension and adequate clearance for the descent of the dumbbell. Eccentric contraction of the elbow and forearm flexors of the non-dominant arm was performed to a count of four seconds. A one second recovery was included where subjects were assisted in return of the dumbbell to the start position (fully flexed elbow). Subjects rested for 30 seconds between each set of ten repetitions and the protocol repeated until subjects failed to complete an eccentric contraction (i.e. controlled descent of the dumbbell). A summary of the exercise protocol for each subject is presented in Table 1.

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Exercise summary for all subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1RM (kg)</td>
</tr>
<tr>
<td>S1</td>
<td>20</td>
</tr>
<tr>
<td>S2</td>
<td>20</td>
</tr>
<tr>
<td>S3</td>
<td>20</td>
</tr>
<tr>
<td>S4</td>
<td>22</td>
</tr>
<tr>
<td>S5</td>
<td>17</td>
</tr>
<tr>
<td>S6</td>
<td>17</td>
</tr>
<tr>
<td>Mean</td>
<td>19.3</td>
</tr>
<tr>
<td>SD</td>
<td>1.9</td>
</tr>
</tbody>
</table>

All subjects recorded pain intensity scores immediately after the exercise protocol. Subjects continued to record heart rate from home in the same manner as Phase A, with the addition of the SF-MPQ each morning and evening until the end of Phase B.
**Data Analysis**

**Data extraction**

Beat to beat (R-R) intervals were extracted from the heart rate monitor using proprietary software (Polar Precision Performance SW, Polar Electro Oy, Finland). Raw R-R interval data was exported into Microsoft Excel for visual inspection. Artifact and ectopic beats were replaced with a beat interpolated between the previous and subsequent normal beats using a method described by the Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology.\(^\text{26}\) Consistent with established guidelines, data was excluded from analysis if ectopy was greater than 5% of the recording.\(^\text{26}\) A clear five-minute period of heart rate data was extracted from the last two thirds of the 15-minute recording to minimise movement ectopy. Normalised data was imported into HRV Analysis Software v1.1 (The Biomedical Signal Analysis Group, Kuopio, Finland). The software transformed normalised data into statistical time domain measures, a Poincaré plot, and frequency spectra quantified by autoregressive and Fast Fourier Transform (FFT) (Figure 2). These measures are widely used in research employing HRV and provide a number of methods to interpret variations in heart rate.\(^\text{26-29}\)
Heart Rate Variability Analysis

RR Interval Time Series

Selected RR Interval Time Series

Time Domain Results

<table>
<thead>
<tr>
<th>Variable</th>
<th>Units</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Statistical Measures</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean RR*</td>
<td>(s)</td>
<td>0.984</td>
</tr>
<tr>
<td>STD</td>
<td>(s)</td>
<td>0.037</td>
</tr>
<tr>
<td>Mean HR*</td>
<td>(1/min)</td>
<td>61.07</td>
</tr>
<tr>
<td>STD</td>
<td>(1/min)</td>
<td>2.45</td>
</tr>
<tr>
<td>RMSSD</td>
<td>(ms)</td>
<td>26.1</td>
</tr>
<tr>
<td>NN50</td>
<td>(count)</td>
<td>51</td>
</tr>
<tr>
<td>pNN50</td>
<td>(%)</td>
<td>5.2</td>
</tr>
<tr>
<td>Geometric Measures</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RR triangular index</td>
<td>(ms)</td>
<td>0.080</td>
</tr>
<tr>
<td>TINN</td>
<td></td>
<td>205.0</td>
</tr>
</tbody>
</table>

Distributions*

<table>
<thead>
<tr>
<th>RRI (s)</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>0.8</td>
<td>0.9</td>
<td>1</td>
</tr>
<tr>
<td>1.1</td>
<td>55</td>
<td>60</td>
</tr>
<tr>
<td>65</td>
<td>70</td>
<td>75</td>
</tr>
</tbody>
</table>

Frequency Domain Results

Non Parametric Spectrum (FFT)

<table>
<thead>
<tr>
<th>Frequency Band</th>
<th>Peak (Hz)</th>
<th>Power (m s^2)</th>
<th>Power (%)</th>
<th>Power (n.u.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>VLF</td>
<td>0.0391</td>
<td>64</td>
<td>11.0</td>
<td></td>
</tr>
<tr>
<td>LF</td>
<td>0.0488</td>
<td>394</td>
<td>67.8</td>
<td>76.2</td>
</tr>
<tr>
<td>HF</td>
<td>0.2402</td>
<td>123</td>
<td>21.2</td>
<td>23.8</td>
</tr>
<tr>
<td>LF/HF</td>
<td></td>
<td></td>
<td></td>
<td>3.205</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Frequency Band</th>
<th>Peak (Hz)</th>
<th>Power (m s^2)</th>
<th>Power (%)</th>
<th>Power (n.u.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>VLF</td>
<td>0.0000</td>
<td>0</td>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td>LF</td>
<td>0.0703</td>
<td>592</td>
<td>83.7</td>
<td>90.4</td>
</tr>
<tr>
<td>HF</td>
<td>0.2363</td>
<td>115</td>
<td>16.3</td>
<td>17.6</td>
</tr>
<tr>
<td>LF/HF</td>
<td></td>
<td></td>
<td></td>
<td>5.141</td>
</tr>
</tbody>
</table>

Poincaré Plot*  SD1 = 18.6 ms ↔ (Short-term HRV)
SD2 = 55.5 ms ↔ (Long-term HRV)

Figure 2  Typical Heart Rate Variability Analysis report
Example HRV Analysis report (subject six, day two) illustrating tachogram of normalised R-R interval data, and transformation of data into statistical time domain measures, a Poincaré plot, and parametric (autoregressive) and non-parametric (Fast Fourier Transform) frequency spectra.

*Results are calculated from the non-detrended selected RRI signal.
For each subject, normalised units (n.u.) of the high frequency (HF) and low frequency (LF) parameters of HRV were taken from FFT, and plotted with pain intensity data (VAS units: mm) against time. For the purpose of visual analysis, the mean of the five baseline data points was calculated for each HRV parameter. The two standard deviation limits were computed from the mean of the baseline data (days 1-5) and added to each plot of HRV versus time. Trend lines, calculated using simple linear regression in Microsoft Excel, were also added to Phase A and Phase B of each graph. Consistent with literature on single-subject designs, visual analysis of changes in level (i.e. above or below the level of the baseline), trend (upward-sloping, downward sloping, stationary), and stability of HRV data between phases was performed.13-15
Results

Six healthy males aged 23 to 36 were recruited into the study. Subject characteristics are presented in Table 2.

Table 2  Subject characteristics

<table>
<thead>
<tr>
<th>ID No.</th>
<th>Age (years)</th>
<th>Occupation</th>
<th>Height (m)</th>
<th>Weight (kg)</th>
<th>BMI (kg/m²)</th>
<th>Routine physical activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>36</td>
<td>Full time student</td>
<td>1.78</td>
<td>70</td>
<td>22.1</td>
<td>Gym, running</td>
</tr>
<tr>
<td>2</td>
<td>30</td>
<td>Full time student</td>
<td>1.75</td>
<td>79</td>
<td>25.6</td>
<td>Yoga, cycling</td>
</tr>
<tr>
<td>3</td>
<td>33</td>
<td>Part time lecturer and land surveyor</td>
<td>1.66</td>
<td>70</td>
<td>25.4</td>
<td>Squash, hiking</td>
</tr>
<tr>
<td>4</td>
<td>29</td>
<td>Part time student and hospitality worker</td>
<td>1.83</td>
<td>95</td>
<td>28.4</td>
<td>Gym</td>
</tr>
<tr>
<td>5</td>
<td>23</td>
<td>Full time winery cellar-hand</td>
<td>1.75</td>
<td>75</td>
<td>24.5</td>
<td>Occupation, tennis</td>
</tr>
<tr>
<td>6</td>
<td>29</td>
<td>Full time student</td>
<td>1.83</td>
<td>74</td>
<td>22.1</td>
<td>Cycling</td>
</tr>
</tbody>
</table>

All subjects completed the 14 day study. Phase A of data collection consisted of daily heart rate recording for five consecutive days, between the hours of 1600 and 2000, to establish baseline HRV. Phase B of data collection consisted of the exercise protocol on day six which was intended to induce DOMS, continued daily heart rate recording, and pain intensity reporting every 12 hours until the end of day 14. Four subjects (S1, S3, S4, and S5) had two days of missing heart rate data. This was due either to excessive artifact, possibly as a result of electromagnetic interference with the heart rate monitor equipment, or from human error. For the purpose of analysis, pain intensity data was subdivided into pre-exercise (day six), immediate post-exercise (day six), pre-DOMS (days 6-7), DOMS period (days 7-9), and recovery period (days 10-14). The Phase B HRV data was subdivided into pre-DOMS (day 6), DOMS period (days 7-9), and recovery period (days 10-14).
Induction of pain associated with DOMS as measured by VAS

Both Figure 3 (High Frequency HRV and VAS versus time) and Figure 4 (Low Frequency HRV and VAS versus time) show pain intensity data for each subject. Pain intensity data was subdivided into pre-exercise (day six), immediate post-exercise (day six), pre-DOMS (days 6-7), DOMS period (days 7-9), and recovery period (days 10-14). The exercise protocol intended to induce DOMS was performed during the morning of day six after collecting pre-exercise pain intensity scores for each subject. Completion of the exercise protocol was determined to be the inability to perform one more eccentric contraction (i.e. control the descent of the dumbbell). For all subjects the pain intensity scores increased immediately after completion of the exercise. In all subjects except one (S4), pain levels then reduced by the evening of day six. During this time S4’s pain intensity score remained the same, although S4 had reported a lower immediate post-exercise score than other subjects.

During the period in which DOMS was expected to occur (days 7, 8, 9), the pain intensity scores increased for all subjects except S5. For S5, pain intensity on day eight (VAS = 5mm) barely rose beyond that seen at the end of day six (VAS = 3mm). One subject, S6, demonstrated a classic DOMS pattern\textsuperscript{16,30} as evidenced by reported pain intensity. The first increase in pain intensity for S6 was seen immediate post-exercise (VAS = 23mm). Pain intensity reduced to zero by the end of day six, indicating full recovery from the exercise protocol. Pain intensity for S6 increased again during day eight (VAS = 42mm), the period in which DOMS was expected to occur. During the recovery period, pain intensity scores for S6 steadily declined to zero over the remaining six days of data collection, indicating full recovery from DOMS.

Delayed onset muscle soreness was also apparent in S1, S2, S3, and S4, although these subjects showed various patterns compared with S6. Subject 4 (S4) reported an injury to the low back/buttock region and non-dominant arm as a result of a fall during the evening of day six, and therefore pain intensity scores appear to show no short-term recovery from the day six exercise protocol. For S2 and S3, the pain intensity scores show a relatively slow recovery rate from the exercise protocol. In S2, the immediate post-exercise VAS score reduced by 50% at the end of day six to
11mm. This score was followed by peak pain intensity one day later on the morning of day seven (VAS = 66mm), the peak of DOMS for S2. In S3, the recovery rate from the exercise protocol was slower, with the immediate post-exercise score reducing by 23% by the end of day seven to a VAS of 47mm. This score was followed by peak pain intensity one day later on day eight (VAS = 62mm), the peak of DOMS for S3.

During the period in which DOMS was expected (days 7, 8, 9), the pain intensity scores for S5 indicate that the exercise protocol did not induce DOMS in this subject. The pain intensity scores for S1 during the expected DOMS period only reached 55% of the immediate post-exercise score of 70mm; however, pain intensity scores indicate delayed soreness during the expected timeframe of 24-48 hours post-exercise. During S1’s recovery period, pain intensity declined to zero then increased again to 23mm on day ten. On day ten, S1 reported mild soreness in the hamstring and oblique abdominal muscles as the result of a routine gym workout on day nine. This increase in pain intensity was likely due to exercise-induced soreness unrelated to the experiment. After day ten, pain intensity for S1 gradually declined to zero towards the end of data collection on day 14 indicating full recovery from muscle soreness either related or unrelated to the experiment.

In summary, the exercise protocol resulted in reports of pain consistent with DOMS in S2, S3, S4, and S6, as evidenced by pain intensity scores. The increase in pain intensity reported by subjects occurred during days 7-9, correlates with literature on the temporal nature of DOMS (i.e. 24-48 hours after the unaccustomed exercise on day six).16-18 The exercise protocol resulted in somewhat less satisfactory DOMS in S1 during days 7-9, and in one subject (S5) DOMS did not occur. As such the high and low frequency HRV data will only be discussed for S1, S2, S3, S4, and S6.

**Normalised high frequency parameter of heart rate variability**

The high frequency (HF) parameter is nominally considered to range between 0.15-0.4 Hz, but also contributes to the low frequency HRV parameter (0.05-0.15 Hz).26 The HF parameter of HRV is clearly established as being dominated by
Parasympathetic activity from the vagus nerve.\textsuperscript{26,31} Parasympathetic activity is known to lower heart rate and the force of cardiac contraction, and increases the beat-to-beat variability of heart rate. A shift towards increased HF in the HRV of a subject indicates increasing parasympathetic activity.\textsuperscript{26,32}

**Phase A – Baseline HF HRV data**

Figure 3 (High Frequency HRV and VAS versus time) shows normalised high frequency HRV data and pain intensity data across Phase A and B for the six subjects tested. In S1 and S6, HF data showed a relatively stable baseline. For S2, HF data during Phase A trends upwards. For S3, Phase A HF data shows a relative trend downwards, but this trend is well contained within two standard deviations of the baseline mean. For S4, baseline data appears unstable. The HF HRV baseline data did not exceed two standard deviations of the baseline mean during Phase A for any of the five subjects.

**Phase B – pre-DOMS, DOMS and recovery period HF HRV data**

In Phase B, HRV data was subdivided into pre-DOMS (day six), DOMS period (days 7-9), and recovery period (days 10-14). In subjects S1 and S6, HF HRV data show no changes during the DOMS period at days 7-9, although S6 showed less stable HF readings from day eight onwards. In S2, an upward-sloping baseline was followed by a level trend line with stable HF data at pre-DOMS into DOMS period. In S3, the downward-sloping baseline levelled off but with more unstable HF readings in Phase B. In S4, HF data showed no distinguishable difference during Phase B, with unstable readings pre- and post-DOMS, and no change in trend.

In those subjects in whom DOMS was achieved (S1, S2, S3, S4, S6), no consistent pattern in the HF data was found that would suggest a relationship between pain associated with DOMS and HRV. In the subject demonstrating a classic DOMS pain pattern (S6), the Phase B HF HRV data showed greater fluctuation compared to baseline and some higher readings, but these readings were not consistently greater than the baseline mean. In three of the five subjects in whom DOMS was achieved
(S3, S4, and S6), the recovery period HF data appeared less stable day to day. In S2, the HF HRV data throughout Phase B was slightly below the baseline mean and level compared to an upward-sloping baseline. Data from S2 also showed increased fluctuation in Phase B data points compared with baseline, but remained stable overall. In S3, the HF HRV data in Phase B was slightly below the baseline mean, showed a slight upward-sloping trend line compared to the downward-sloping baseline, and had greater fluctuation and instability compared to baseline. In summary, changes to HF HRV data could not be related to pain intensity in the five subjects in whom DOMS was satisfactorily achieved.
Figure 3  High Frequency HRV and VAS versus time. Scatter plot representing five minutes of normalised HF HRV data collected between the hours of 1600 and 2000 over 14 days, with trend lines added to Phase A and Phase B HRV data. Line graph across days 6-14 representing VAS data collected pre-exercise, immediate post-exercise, end of day six and every 12 hours from day 7-14. Baseline mean calculated from day 1-5 HF HRV data points. Four subjects (S1, S2, S4, S5) have one HF HRV data point missing due to human error, and one data point excluded due to ectopy exceeding 5%. 

<table>
<thead>
<tr>
<th>Key:</th>
<th>Phase A HRV</th>
<th>Phase B HRV</th>
<th>Exercise protocol – day six</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline mean</td>
<td>2SD</td>
<td>VAS</td>
</tr>
</tbody>
</table>

N Phase  HRV  N BN  NBN  N Exercise protocol –  N N NN
day six NN

Figure 3  High Frequency HRV and VAS versus time. Scatter plot representing five minutes of normalised HF HRV data collected between the hours of 1600 and 2000 over 14 days, with trend lines added to Phase A and Phase B HRV data. Line graph across days 6-14 representing VAS data collected pre-exercise, immediate post-exercise, end of day six and every 12 hours from day 7-14. Baseline mean calculated from day 1-5 HF HRV data points. Four subjects (S1, S2, S4, S5) have one HF HRV data point missing due to human error, and one data point excluded due to ectopy exceeding 5%.
Normalised low frequency parameter of heart rate variability

The low frequency (LF) parameter of HRV is nominally considered to range between 0.05-0.15 Hz. Studies indicate that the LF parameter of HRV is mediated by both sympathetic and parasympathetic activity to the heart. Baroreceptor activity is thought by some to be the predominant influence on LF, although data from animals and humans suggests a medullary origin. Since LF is not specific for either ANS division it is usually not considered a general index of sympathetic cardiac control or sympato-vagal balance.

Phase A – Baseline LF HRV data

Figure 4 (Low Frequency HRV and VAS versus time) shows normalised low frequency HRV data and VAS data across Phase A and B for the six subjects tested. In S1 and S6, LF data showed a relatively stable baseline. For S2, LF data during Phase A trends downwards. For S3, Phase A LF data shows a relative trend upwards, but this trend is well contained within two standard deviations (2SD). For S4, baseline LF data appears unstable. The LF HRV baseline data did not exceed 2SD during Phase A for any of the five subjects.

Phase B – pre-DOMS, DOMS and recovery period LF HRV data

In Phase B, HRV data was subdivided into pre-DOMS (day six), DOMS period (days 7-9), and recovery period (days 10-14). In S6, LF HRV data showed a drop during day eight (i.e. within the DOMS period) that exceeded 2SD, followed by less stable LF readings from day eight onwards. For S2, a downward-sloping baseline was followed by a level trend line with stable LF data at immediate post-exercise and pre-DOMS into the DOMS period. In S3, the upward-sloping baseline levelled off during Phase B, and the data was less stable overall than baseline. In S4, LF data showed no distinguishable difference during Phase B, with unstable readings pre- and post-DOMS, and no change in trend.

In those subjects in whom DOMS was achieved (S1, S2, S3, S4, S6), no consistent pattern in the LF data was found that would suggest a relationship between pain
associated with DOMS and HRV. In the subject demonstrating a classic DOMS pain pattern (S6), the Phase B LF HRV data showed greater fluctuation compared to baseline and some lower readings, but these readings were not consistently lower than the baseline mean. In three of the five subjects in whom DOMS was achieved (S3, S4, and S6), the recovery period LF data appeared less stable day to day than the pre-DOMS period. In S2, the LF HRV data throughout Phase B was slightly above the baseline mean and level compared to a downward-sloping baseline. Data from S2 also showed increased fluctuation in Phase B data points compared with baseline, but remained stable overall. In S3, the LF HRV data in Phase B was slightly above the baseline mean, showed a slight down-slope compared to an upward-sloping baseline, and had greater fluctuation and instability compared to baseline. In summary, changes to LF HRV data could not be related to pain intensity in the five subjects in whom DOMS was satisfactorily achieved.
Figure 4  
Low Frequency HRV and VAS versus time. 
Scatter plot representing five minutes of normalised LF HRV data collected between the hours of 1600 and 2000 over 14 days, with trend lines are added to Phase A and Phase B HRV data. Line graph across days 6-14 representing VAS data collected pre-exercise, post-exercise, end of day six and every 12 hours from day 7-14. Baseline mean calculated from day 1-5 LF HRV data points. Four subjects (S1, S2, S4, S5) have one LF HRV data point missing due to human error, and one data point excluded due to ectopy exceeding 5%.
To summarise, the exercise protocol produced muscle failure in all six subjects, that is, inability to perform one complete eccentric contraction. The exercise protocol also increased the level of pain in all subjects, as evidenced by immediate post-exercise pain intensity scores. Pain intensity scores for all subjects, except S4, declined by the end of day six, although the rate of recovery varied between subjects. During the expected DOMS period (day 7-9), the level of greatest pain intensity was variable between subjects. During the recovery period (days 10-14), the DOMS recovery rate was also variable as S1, S3 and S4 did not have a steady decline in pain intensity, such as that seen in S2 and S6.

Using visual analysis, there was no correlation between the Phase B pain intensity scores and changes to the normalised low and high frequency HRV parameters in the six subjects tested. Although slight differences were observed in the stability of HRV data and slope and level of trend line with some subjects, no consistent pattern of HF or LF variation emerged that can be clearly attributed to DOMS. In conclusion, any effect from DOMS on HRV was not visually identifiable from the results obtained at the timeframes measured.
Discussion

The aim of this study was to determine whether the physiological effects of delayed onset muscle soreness (DOMS), as a model of mild to moderate musculoskeletal pain, would influence the autonomic nervous system (ANS) as measured by heart rate variability (HRV). Heart rate data from six subjects was collected from baseline through to the pre-DOMS period and from the DOMS period through to full recovery. Pain intensity data was collected pre-exercise and immediate post-exercise, then throughout the pre-DOMS, DOMS, and recovery periods. Delayed onset muscle soreness occurred in 5 out of 6 subjects tested, as evidenced by pain intensity scores, although visual assessment failed to reveal any consistent pattern between DOMS and HRV. The results indicate that ANS changes did not occur after induction of DOMS in the five subjects at the timeframes measured. Although ANS changes were evident, as indicated by changes to HRV both before and after onset of DOMS, these changes are more likely attributable to uncontrolled factors such as individual physiological and environmental variation rather than DOMS.

Outcomes

Delayed onset muscle soreness as a model of pain

Pain is recognised to be a complex phenomenon involving both peripheral mechanisms and higher centre involvement.\textsuperscript{37} The experience of pain can be classified into sensory and affective dimensions, that is, physiological or psychological elements.\textsuperscript{38} Delayed onset muscle soreness is an effective model of pain in terms of the sensory dimension. For example, the mechanisms underlying DOMS are purported to involve micro-damage to tissue and stimulation of free nerve endings from the breakdown products of tissue damage,\textsuperscript{30} with a subsequent inflammatory response involving substances such as bradykinin which are known to stimulate free nerve endings.\textsuperscript{39} In terms of the affective or psychological dimension of pain, DOMS would, like other noxious stimuli, be dependant upon aspects such as cognitive appraisal of threat cues,\textsuperscript{40,41} environmental context,\textsuperscript{42} coping strategies,\textsuperscript{43} and prevailing emotion (e.g. fear or anxiety).\textsuperscript{44} Previous studies have successfully
employed DOMS to examine the role of various affective components of pain, such as fear of pain\textsuperscript{45} and the role of recalled, expected, and actual pain.\textsuperscript{46}

The pain intensity scores in this study indicate that the exercise protocol was, for the most part, effective in inducing DOMS, and that DOMS was an effective model of pain. Completion of the exercise protocol was defined as the inability to perform one complete eccentric contraction, at which point it was assumed that muscle failure was achieved. Although the exercise protocol produced muscle failure in all subjects, the protocol induced a pattern of pain in two subjects which was not a “classic” DOMS pattern.\textsuperscript{17,47} These two subjects (S1 and S5) appeared to have more upper-body exercise in their routine physical activities than other subjects: S1 performed pull-ups as part of a regular gym routine and S5 had a labour intensive occupation (winery cellar-hand). Muscle failure was achieved in these subjects at the time of the exercise intervention, yet, they reported less pain during the DOMS period (24-48 hours post-exercise) than the immediate post-exercise period. The routine physical activities of S1 and S5, coupled with the apparent minimal DOMS, suggests that the elbow and forearm flexor muscles may have been more preconditioned due to the repeated-bout effect. The repeated-bout effect occurs when subsequent bouts of eccentric exercise, from 1-10 weeks after the initial bout, produce much less muscle damage – implying an adaptive and protective function.\textsuperscript{48,49}

Such preconditioning of S1 and S5 may have contributed to the apparently minimal DOMS produced. Also of consideration, however, is that the reported intensity of DOMS soreness has been found to have poor correlation with objective markers of muscle damage.\textsuperscript{50,50} Differences in reported DOMS intensity have been attributed to factors such as natural variation in muscle stiffness and static flexibility,\textsuperscript{51} and genetic differences in muscle fibre type and response to damage.\textsuperscript{52} This variation may provide an explanation for the difference in reported DOMS pain intensity amongst subjects of the current study, particularly S1 and S5. Although the manner in which DOMS was induced was consistent with previous studies,\textsuperscript{16-18} DOMS may have been more clearly established in all subjects with a more rigorous exercise protocol, such as a dumbbell greater than 40-50% of the 1RM, or a protocol comprising both concentric and eccentric exercise. Similarly, more thorough pre-screening of subjects may have
excluded candidates who were likely to be less susceptible to DOMS of the elbow and forearm flexor muscles.

**Heart rate variability**

Independent of DOMS, the baseline high and low frequency HRV data showed inter-subject differences. Some subjects recorded more stable HRV data than other subjects did. Immediate post-exercise, pre-DOMS, DOMS, and recovery period data also showed these inter-subject differences. For each subject, stability was variable from baseline through to recovery, with apparent trends occurring in some cases – but this was prior to, rather than a result of, DOMS. Despite these observations, no consistent pattern was evident that would suggest any correlation between HRV and the reported pain associated with DOMS. The lack of consistent pattern highlights the multitude of factors that are potentially involved when examining HRV data. When methods of HRV analyses first emerged as a clinical tool, both researchers and clinicians viewed it as a simple non-invasive technique to assess cardiovascular status. Current literature is clear that data from HRV is more complex than originally thought, as reflected in the rapidly evolving quantitative approaches to HRV, and the numerous investigations examining the overlapping linear and non-linear factors that influence HRV. These influences include posture, mental stress, neuroendocrine and circadian processes, respiration, and ageing.

In the present study, a pattern that would suggest a correlation between DOMS and HRV would be a clear shift toward the HF parameter during the DOMS period (24-48 hours post-exercise). A shift towards HF would have indicated increased parasympathetic activity in subjects during that timeframe. By comparison, the HRV in the same subjects without DOMS, would not display this pattern and only show normal variation related to respiration and to processes such as circadian rhythms or factors such as posture. This comparison is highly simplified however, as the HRV of healthy subjects is remarkably complex, and caution should be applied when interpreting all HRV data as it is dependent on factors such as age and gender, serum lipoproteins and leukocytes, and lifestyle factors (for a comprehensive account see the 1996 Task Force review). The results of the current
study do not appear to demonstrate a pattern that indicates a shift towards one or other HRV parameter. Additionally, identification of proportional changes to HF and LF in the present study was problematic due to difficulty representing five baseline data points against 14 post-intervention data points.

The low frequency (LF) parameter of HRV represents both sympathetic and parasympathetic activity to the heart. The high frequency (HF) parameter of HRV represents parasympathetic activity mediated by the vagus nerve. As vagal activity governs respiration, HF is also described as the ‘respiratory frequency’. The term respiratory sinus arrhythmia (RSA) describes spontaneous fluctuations in heart rate from respiratory-driven vagal modulation of sinoatrial node activity. These fluctuations result from central coupling between respiratory oscillators in the brainstem and autonomic outflow. Heart rate variability is largely dominated by ANS activity, however, HRV is an indirect measure of mean autonomic tone. For example, HRV data reflects the state and function of the sinoatrial node, humoral factors, and central heart rate oscillators such as the respiratory centre of the brainstem. Autonomic activity, therefore, is not the sole influence on data obtained from HRV. A more direct, yet invasive measure, such as microneurography, would provide a more accurate representation of ANS activity on heart rate but was not practical for this preliminary study.

Though HRV is an indirect measure of autonomic activity, any potential effect from DOMS on the ANS may have been of insufficient magnitude to affect HRV. For example, any change to local physiology from DOMS may have potentially caused changes in local autonomic fibres, however these changes may have not been potent enough to potentiate changes in autonomic fibres beyond the perturbed area. In this type of scenario, any DOMS-induced ANS change would have negligible effect on HRV.
Related research

Although there are no previous studies investigating the effect of DOMS on ANS indices, there is related research that indicates a possible relationship between the ANS and injured muscular or articular structures in humans. For instance, Grimm, Cunningham, and Burke\(^7\) published a case series comparing ANS indices in acute injured subjects and healthy controls. Grimm et al.\(^7\) concluded that “skin conductance was statistically greater in the musculoskeletal injury group than in the control group” and that analysis showed “a highly significant positive relationship between skin conductance and SBP_{LF} [systolic blood pressure low frequency]”. The greater skin conductance and higher SBP_{LF} in the acute injured group signifies increased activity of cutaneous sympathetic neurons and increased activity of vasomotor sympathetic neurons respectively. Grimm\(^7\) surmised that an interaction exists between cutaneous and vasomotor sympathetic neurons in those with acute musculoskeletal injury, and that this interaction manifested as altered cardiac and peripheral autonomic function. Yet, Grimm\(^7\) found no difference in the low and high frequency variables between the injured group and controls, despite the presence of smaller standard deviations in the injury group.

Various limitations within the investigation by Grimm et al.\(^7\) highlight the need for caution when interpreting their results. To illustrate, heterogeneity from age and sex-matched controls imparts a considerable amount of individual variation when comparing ANS indices in the two groups. In addition, heterogeneity in the injured group (e.g. site, duration, and intensity of injury), may have potentially introduced a number of variables. These include psychological variables associated with low back pain,\(^69-71\) physiological variables associated with density of pain sensitive free-nerve endings in different tissues (e.g. the meniscotibial ligament of the knee versus the medial collateral ligament of the knee),\(^72,73\) or variables attributed to central sensitisation mechanisms associated with injuries of longer duration (e.g. 24 hours versus six days).\(^74\) In addition, it is possible that the “routine chiropractic physical examination” given to the injured group and not the control group, biased Grimm’s\(^7\) results. For example, the spinal and cutaneous stimulation during the examination may have influenced the ANS prior to data collection, although it is difficult to
predict how the stimulation would have manifested and how long such influence may have persisted.

In the present study, the results do not clearly indicate that DOMS produced an effect on the ANS via HRV. As autonomic activity and pain are highly complex and individual, there may be a number of influences, both physiological and cognitive, that may have been a factor in this study. The following section discusses these influences.

**Cognitive and physiological influences on the ANS and on pain**

The study employed DOMS in an attempt to simulate a naturally occurring soft tissue injury. It would be fair to assume that unlike DOMS, naturally occurring injuries are not restricted to damage to one tissue type (e.g. myofascia), may be sudden in onset, and may have a more threatening context overlaying the injury. Cognitive factors, such as threat of harm or injury, can have a significant effect on the ANS. In the present study, cognitive factors may have played a greater role than anticipated. Research has shown that fear, threat value, attention, control, predictability, information, environmental cues, emotion and context all influence perception of pain and evocation of autonomic responses. Threat value in particular has a profound effect on pain. Several features of the present investigation provide examples of lack of threat. To illustrate, the DOMS information sheet [see Appendix D] included information on the safety of DOMS. Recently, the effect of ‘safe’ and ‘threatening’ cues in a situation involving a potential source of pain (e.g. electric shock) has been found to have a considerable influence on autonomic indices. Bradley, Silakowski, and Lang found that “All subjects reacted with greater defensive reactivity, including potentiated startle blinks, heightened skin conductance, and cardiac deceleration in the context of threat, compared to safe, cues.” In the present study, the content of the DOMS information sheet may have provided sufficient ‘safe’ cues to subjects to reduce threat value. For example, the impending discomfort was likened to soreness commonly encountered after over-exertion, was described as short-lasting and not associated with damage to tissue other
than muscle, and was currently believed by researchers to confer a protective effect due to muscle adaptation.

Inducing DOMS in the non-dominant arm, which is used less frequently in activities of daily living, may have likewise reduced threat. By comparison, the investigation by George, Dover, and Fillingim\textsuperscript{45} induced DOMS in the shoulder muscles of the dominant arm, which may have been sufficiently threatening to subjects to generate pain-related fear. This fear had a greater influence on pain perception and fear of movement, than factors such as anxiety, gender, and catastrophising. Comparing the present study to the Grimm et al.\textsuperscript{7} investigation, DOMS was a relatively homogeneous tissue damage, which could have lessened threat value to subjects. Grimm’s data may have been influenced by greater threat, as subjects had naturally occurring injuries possibly involving heterogeneous tissue damage (e.g. knee injury, shoulder injury), which may have also been acquired under more threatening circumstances.

Another cognitive aspect to consider in the present study is that previous experiences of pain can affect the relationship between expected and actual pain responses.\textsuperscript{82} Dannecker, Price and Robinson\textsuperscript{46} have reported similar findings to several other investigations\textsuperscript{83-86} in that the expectation of pain intensity and unpleasantness correlated strongly with subsequent pain reports. Investigators are still elucidating the relative influence of expectation and actual pain on recalled pain; however, it is conceivable that in the present study, the recall of previous DOMS experiences may have influenced the expectation of DOMS intensity, and thus pain report. The influence of recalled, expected, and actual pain may have also played a role in the Grimm et al.\textsuperscript{7} investigation, as a naturally acquired injury may have been more painful, and possibly more threatening, with little or no previous experience of the tissue damage (e.g. knee injury in a previously untraumatised knee).

Threat is strongly linked with context.\textsuperscript{40,75,87} The experimental environment of the present study (gym and home) was familiar to subjects, and thus likely interpreted as being ‘safe’. This contrasts with the unfamiliar “private and thermo-controlled autonomic laboratory” of the Grimm et al.\textsuperscript{7} investigation, which may have influenced ANS indices (e.g. greater skin conductance) more than the present study. The experimental environment of the present study also included stimuli from a nature
documentary. Research has demonstrated that environmental factors such as lighting, colour, and auditory stimuli, visual distraction, and exposure to scenic imagery impact on patient recovery and pain thresholds and tolerance. Although results differ in the literature, and the underlying mechanisms are not conclusive, scenic, pleasant, and unpleasant visual stimuli affect autonomic indices. Based on such research, it is reasonable to suppose that the visual and auditory stimuli provided by the nature documentary, being distracting and scenic, might have influenced the ANS and thus HRV during heart rate recording. The visual and auditory stimuli may have also influenced respiratory rate, modulating RSA and thus the HF parameter of HRV. Other studies using HRV have included controlled breathing to restrict RSA. Due to the field nature of the present investigation, it was not feasible to include controlled breathing during data collection.

A further element associated with threat is that of predictability. According to Rhudy and Meagher, predictable yet unavoidable shock produces fear, which evokes the release of endogenous opioids and attenuates pain. In contrast, the possibility of shock results in apprehensive anticipation of a potential threat, which can facilitate increased sensory receptivity and enhance pain. The anticipation of threat in the absence of actual exposure is enough to potentiate startle reflexes and autonomic responses. In the present study, it is possible that the anticipation of pain associated with DOMS was lessened, as subjects were made aware of the temporal aspect of DOMS during recruitment. Conversely, the pain associated with DOMS was also predictable yet unavoidable, which too may have influenced pain perception and pain report.

Aside from cognitive influences, normal physiological processes could have played a greater role in the current study. Given that HRV is an indirect measure of the ANS, the effect on HRV from processes such as circadian rhythms and normal hormonal fluctuation may have been of greater magnitude than an effect, if any, from DOMS. To illustrate, baseline HRV data was very similar in some subjects to the pre-DOMS and DOMS period data, indicating little overall fluctuation in HRV from DOMS, even though small differences on certain days may have been evident. Increased data point density, such as 10 minute Holter recordings taken hourly for 72 hours during the expected DOMS period, may have provided a more accurate
estimation of whether DOMS influenced HRV. This is a recommendation for any future investigations.

The normal physiological response to the exercise protocol performed on day six may have also played a role. Pober, Braun and Freedson\textsuperscript{106} have found that even a single bout of exercise produced changes in HRV similar to that seen in studies on long-term training. The authors suggest that the changes in autonomic function observed with a single bout of exercise indicate a shift towards a more stable autonomic environment for the heart. In the present study, this effect may have potentially been relevant to the data, as HRV would shift toward HF after the exercise protocol with such an effect. Unfortunately, however, it is uncertain how long any proposed effects on HRV from a single bout of exercise might have persisted (e.g. immediate post-exercise through to DOMS). Increased density of data points from the post-exercise period through the DOMS period may have shown more clearly whether this effect was relevant.

If the physiological changes associated with DOMS do have local or wider effects on the ANS, the age and good health of the subjects may have compensated for any potential changes. For example, it is reasonable to infer that the proposed mechanisms underlying DOMS (micro-damage to muscle tissue and subsequent inflammation) may have been less challenging to the physiology of a young healthy subject than tissue damage that was more severe or heterogeneous. To illustrate, the HRV of a young healthy person is likely more capable of compensating for any physiological challenge than that of an older person, or one with limited cardiovascular adaptability.\textsuperscript{107,108} Such compensation may have been evident in one subject of the present study (S4), who sustained a naturally-acquired injury during the end of day six, the pre-DOMS period. No difference in the pre-DOMS and DOMS period HRV was observed in this subject, possibly demonstrating the greater compensation capacity present in a healthy young person.
Limitations and weaknesses

This study has several limitations and weaknesses. The design consisted of five baseline HRV data points followed by nine post-intervention data points. Five baseline data points is the minimum recommended in literature on single-subject research designs. A greater number of baseline HRV data points (minimum eight) would have enhanced the ability to define the baseline and to evaluate any changes observed after induction of DOMS, particularly proportional change of HRV parameters. Greater frequency of measurement would have also benefited analysis of the DOMS period (24-48 hours post-exercise). Increased frequency of heart rate recording (e.g. Holter monitoring) during the DOMS period (days 7-9) would have enhanced the ability to discern any effect of DOMS on the ANS, if any, as DOMS is temporal and the effect on the ANS likely not persistent.

The ECG equipment (‘heart rate monitor’) used in this study has shown good reliability and validity. In contrast, the stability of short-term (5-15 minutes) HRV recordings has shown highly variable reliability coefficients in several investigations (for review see Sandercock et al.), and the poor reliability of HRV found in previous investigations may be a factor in this study. Another limitation was that the recording environment of the present study was under limited control, therefore influences on HRV from factors such as posture and head-tilt may have been present during recording sessions. Similarly, the field setting of the study limited the ability to inspect and monitor ECG signal quality, or subjects’ operation of the heart rate monitor. These factors may have contributed to the ectopy experienced, despite attempts to control for similarity of recording conditions, time of recording, and extrinsic influences such as stimulant consumption.

Pain intensity scores during baseline were not collected from subjects: subjects were pain-free at recruitment and were assumed to remain pain-free throughout the baseline period. This data may have been useful to compare baseline pain intensity with baseline HRV. Nonetheless, repeated administration of a pain-related questionnaire to pain-free subjects during the baseline period could conceivably have lead to heightened ‘somatic focus’, and to higher retest scores. The term somatic focus describes the tendency to attend to and report somatic symptoms, that is, physical
sensations, which do not correlate with objective measures of health status.\textsuperscript{113, 114} Correlations have been found between increased somatic focus and pain report in a variety of populations, which potentially could include subjects of the current study. These populations include younger age groups, those with history of abuse and affective disorders, and those of lower socioeconomic status.\textsuperscript{115-118}

Following convention on single-subject research designs, the current study used visual analysis of results, such as trend, slope, and fluctuation.\textsuperscript{13-15} This method has intrinsic strengths and weaknesses. Regarding intervention effects, some argue that visual analysis emphasises clinical rather than statistical significance, has a low error rate, and identifies treatment effects more conservatively.\textsuperscript{119-121} Yet, there are no formal rules regarding interpretation of data and others contend that the poor reliability of visual analysis weakens rigor and the ability to determine causality.\textsuperscript{119, 122, 123}

Use of inferential statistical analysis may have been a beneficial adjunct in terms of increased sensitivity towards interpreting any potential effect DOMS may have had on HRV. However, like many single-subject designs, it is probable that the present study failed to meet parametric assumptions (e.g. normality, equal variance, and serial independence). Kazdin\textsuperscript{14} states that statistical analysis may be of value in single-subject research when there is no stable baseline, the effects cannot be well predicted, and statistical control may be required for “extraneous factors in naturalistic environments” – features which are evident in the current study. A number of authors echo Kazdin’s sentiment, and spirited debate has occurred between proponents of both visual and statistical analyses regarding sensitivity and what constitutes a meaningful change, and in particular the phenomenon of autocorrelation.\textsuperscript{119, 120, 124-126} Autocorrelation is a statistical term referring to the correlation of a time series with its own past and future values.\textsuperscript{119, 123} Such correlation complicates use of inferential statistical analysis as it violates both parametric and non-parametric assumptions. Statistical methods that do not account for any trend-related autocorrelation lowers reliability by distorting effect size magnitude, confidence intervals, and \( p \)-values, regardless of study design.\textsuperscript{119, 123, 124}
For future investigations, measuring a variety of autonomic indices, such as HRV, edge-light pupil cycle time, and galvanic skin response may be of benefit. Similarly, larger sample sizes and a different design may also be advantageous. For example, a prospective design using naturally occurring soft tissue injuries with similar characteristics (e.g. site, intensity, duration) could be useful, although this design has inherent logistical challenges. A cross-sectional design with matched controls may also provide more robustness than the present study.
Conclusion

The results of this study indicate that changes to HRV did not occur as result of pain associated with DOMS within the measurement timeframe employed. Although slight differences in the stability of HRV data and slope of trend line occurred in some subjects, no consistent pattern emerged that was attributable to DOMS. If DOMS was able to influence the ANS, the effect on HRV appeared negligible. Furthermore, changes to HRV parameters that were evident, were likely attributable to various physiological and cognitive factors that are known to influence pain perception and autonomic indices.

To date, the relationship between pain and the ANS has not been fully established: for example, noxious stimuli clearly and consistently modulates autonomic activity yet the autonomic effects from mild to moderate pain are less consistent. Scientific studies to date do not support this osteopathic concept, and there is a scant amount of literature addressing this issue. Construction of this study was motivated by a tenet presented within many osteopathic texts, namely, that musculoskeletal or visceral disturbance can have effects elsewhere in the body via the ANS. Scientific studies to date do not support this osteopathic concept, and there is a scant amount of literature addressing this issue. The results of the current study, whilst preliminary, did not provide supportive evidence for this concept, and indeed may even challenge it. There have been calls in the literature for a definitive illustration of whether musculoskeletal or visceral ‘disturbance’ could have a deleterious impact on the ANS, and potentially on health and wellbeing. Establishing the nature and significance of these purported links is important for evaluating tenets within osteopathy, and may be of clinical relevance to those with acute injury, or those in chronic pain states.
Acknowledgments

Thanks are extended to Mr Darren Lyne for providing the nature documentary DVD’s used in this project.
References


23 Price DD, McGrath PA, Rafii A, Buckingham B. The validation of visual analogue scales as ratio scale measures for chronic and experimental pain. Pain 1983; 17: 45-56.


119 Brossart DF, Parker RI, Olson EA, Mahadevan L. The relationship between visual analysis and five statistical analyses in a simple AB single-case research design. Behav Modif 2006; 30: 531-63.
Parker RI. Increased reliability for single-case research results: is the bootstrap the answer? *Behav Ther* 2006; 37: 326-38.


Appendix A: Subject consent form

CONSENT FORM

Muscle Soreness and Heart Rate Project

This research project investigates the effects of muscle soreness on heart rate. The research is being undertaken by Larissa Morgan from Unitec New Zealand, and will be supervised by Robert Moran and Dr Craig Hilton.

Name of Subject: …………………………………………………………………………..

I have seen the Information Sheets dated ……………………………………………for people taking part in the Muscle Soreness and Heart Rate Project. I have had the opportunity to read the contents of the two information sheets and to discuss the project with the researchers and I am satisfied with the explanations I have been given. I understand that taking part in this project is voluntary (my choice) and that I may withdraw from the project any time up until the point of data analysis (1 week after the last day of scheduled data collection) and this will in no way affect my access to the services provided by Unitec New Zealand or any other support service.

I understand that I can withdraw from the research if, for any reason, I want this.

I understand that my participation in this project is confidential and that no material that could identify me will be used in any reports on this project.

I have had enough time to consider whether I want to take part.

I know whom to contact if I have any questions or concerns about the project.

The principal researcher is Larissa Morgan [ph: (09) 815-4321, e-mail: morgal02@studentmail.unitec.ac.nz]

Signature…………………………………………………………………………………..subject …………..(date)

Project explained by……………………………………………………………………

Signature…………………………………………………………………………………..(date)

This study has been approved by the Unitec Research Ethics Committee from 26 July 2006 to 31 December 2007. If you have any complaints or reservations about the ethical conduct of this research, you may contact the Committee through the Chairperson (ph: 09 815-4321 ext 8384). Any issues you raise will be treated in confidence and investigated fully, and you will be informed of the outcome.
Appendix B: Subject information sheet

INFORMATION SHEET

Muscle Soreness and Heart Rate Project

About this research
You are invited to take part in a research project being undertaken as part of the completion requirements of a Masters of Osteopathy degree.

This research investigates the effects of eccentric exercise of the non-dominant arm on nerve activity (namely heart rate) in males aged 18-40.

Participation in this project will require:

- Signing a consent form
- Attendance at an initial screening appointment for collection of health information (e.g. smoking history, age, and spinal/cardiac/respiratory or neurological problems, disease or history of surgery) to establish your suitability for participation in the research.
- Removal of your shirt and placement of a chest strap and wristwatch (this will be loaned to you to record heart rate). The use of the watch will be fully demonstrated to you. In the experiment you will set these up for yourself. The researchers will be available by phone to assist you with setting these up if you experience any technical problems.
- Demonstrate that you can confidently operate the watch and correctly locate the chest strap.
- Demonstrate comprehension of daily protocol for data collection.
- Setting aside a consistent time in the same venue (e.g. home) for 14 consecutive days, for 15 minutes each day at a time specified. During this time you will need to wear the heart rate monitor and transmitter chest strap, while watching a nature documentary that will be provided to you on DVD or videotape.
- Being available to attend Unitec for 30 minutes to perform an exercise protocol on Day 6 of data collection that will induce muscle soreness (see attached Information Sheet).
- Consent to the research teams use of research data in preparing both a research project dissertation and an article for publication (all data will be anonymised)

Your involvement in this research will aid the understanding of the human body’s response to eccentric exercise.

The Researchers
The primary researcher is Larissa Morgan
This project is being supervised by Robert Moran and Dr Craig Hilton
Participation and Consent

We would greatly appreciate your participation in the study. If you would like to participate please complete a consent form for this project and return it to Larissa Morgan.

You have the right to not participate. If you consent but later wish to withdraw from this research, you may do so at any time up until the point of data analysis (1 week after the last day of scheduled data collection). This can be done by phoning us, or by telling us when we contact you that you do not want to participate.

Getting help

Please contact either one of us should you require help with anything regarding this research project.

Larissa Morgan  E-mail morga102@studentmail.unitec.ac.nz
 Ph  (09) 815 4321 x8642

Robert Moran  E-mail rmoran@unitec.ac.nz
 Ph  (09) 815 4321 x8642

Dr Craig Hilton  E-mail chilton@unitec.ac.nz
 Ph  (09) 815 4321 x8601

Information and concerns

If you want further information about the project you can call, or email the above address. At anytime if you are concerned or confused about the research project you may contact Larissa Morgan the primary researcher using the contact details above.

If you have concerns about the way in which the research is being conducted you can contact the following:

Health Advocates: Advocates Network Services Trust, Phone (09) 623 5799, 0800 205 555, Fax (09) 623 5798, PO Box 9983, Newmarket, Auckland.

Confidentiality

Confidentiality and your anonymity will be protected in the following ways:

- Information and data collected from you during the research will be labelled with an identification number for the sole purpose of anonymously comparing your data between subsequent sessions.

- All computer records will be accessible only by passwords held only by the researchers.

- Any data derived from the research will be anonymised and your identity will be kept confidential.

A copy of the final report will be available at Unitec New Zealand library. All subjects are welcome to view this. Summaries and recommendations may be published in research journals.

Finally, we would like to thank you for your valuable contribution to this research.

This study has been approved by the Unitec Research Ethics Committee from 26 July 2006 to 31 December 2007. If you have any complaints or reservations about the ethical conduct of this research, you may contact the Committee through the Chairperson (ph: 09 815-4321 ext 8384). Any issues you raise will be treated in confidence and investigated fully, and you will be informed of the outcome.
Appendix C: Operational instructions

Instructions for Heart Rate Recording

Please remember: no caffeine, alcohol, food or exercise 3 hours prior to recording. Caffeine is found in coffee, tea, and energy drinks with guarana such as red bull, V, lift plus, e2.

Familiarise yourself with the instructions for the first couple of days

CHEST STRAP TRANSMITTER
1. Moisten the 2 electrode areas at each end of the strap under running water, and make sure they are well moistened.
2. Position the letter L on the plastic connector with the word LEFT on the strap, and fasten.
3. Loop the strap around your chest (under your clothes) and position against your skin - fasten the RIGHT side of the strap to the plastic connector
4. Adjust the strap length so that it fits snugly yet comfortably
5. Position the plastic connector at the centre of the lower end of your sternum (breast bone). The strap should be just below your pectoral muscles and the connector in a central and upright position.
6. Check that the wet electrode areas are firmly against your skin
7. Put wristwatch on

PREPARE TO WATCH THE DVD
1. Remove the potential for distractions, i.e. telephones & mobiles, pets, T.V., music/radio, children.
2. No food or drink is to be taken at this time
3. Insert the DVD and position yourself comfortably in a chair (use the same chair and same seated position each day)
4. Make sure wristwatch is less than 1 metre from the chest transmitter
5. Once the documentary begins, wait approximately 2 minutes before starting heart rate recording

HEART RATE RECORDING USING THE WRISTWATCH
1. Press the red start button
2. The wristwatch will search for the transmission from the chest strap
3. Once the transmission is found the watch will display a ‘beating heart’ icon
4. Press the red start button again to begin recording, a stop-watch timer will now display
5. Watch the DVD for approximately 15 minutes, keeping movement to a minimum
6. Press the stop button (lower left on watch) to stop recording
7. Press the stop button again to return the watch back to normal time and date display. Please note: your heart rate and other physiological data cannot be accessed from the wristwatch itself without the appropriate software and uplink unit.
8. Unfasten the chest strap transmitter and remove the plastic connector from both ends of the strap
9. Store the strap, connector, and watch in the separate bags provided to prevent moisture from affecting the components.
10. Fill in the diary/comments sheet noting any stressful incidents, accidents, injuries, or illnesses that may have occurred or been present that day, or any necessary medication that may have been taken during the day.
Appendix D: DOMS information sheet

INFORMATION SHEET

Delayed Onset Muscle Soreness

What is delayed onset muscle soreness?

Delayed onset muscle soreness (DOMS) is the sensation of dull aching pain and discomfort felt in the muscles after having done unaccustomed or strenuous exercise.

Why does DOMS come about?

DOMS particularly occurs after having done exercise that involved the muscles lengthening whilst trying to resist or control a load – otherwise known as an eccentric contraction. An example of eccentric contraction is found in walking down hill: the quadriceps muscle contracts to control the rate of knee flexion against gravity as we step down the slope, and lengthens during the process. Another similar example is found in straightening a bent arm when holding a heavy shopping bag: the biceps is lengthening whilst controlling the rate of lowering the bag.

When do I feel DOMS?

One of the key features of DOMS is, as the term implies, the delayed onset of discomfort. There is no discomfort immediately after the exercise or activity as DOMS typically develops several hours afterwards, peaking 24-48 hours later and disappearing completely by 3-7 days. The time frame of DOMS distinguishes it from temporary soreness which is experienced during the final stages of muscle fatigue and which is thought to be caused by metabolic waste accumulation (e.g. lactic acid).

How does DOMS happen?

The precise physiological mechanism behind DOMS is not yet fully understood, however there are currently two well-established and accepted theories:

1. the loading on the muscle from the eccentric contraction disrupts and strains microscopic components of the muscle
2. a subsequent inflammatory reaction to the cellular disruption

Will DOMS affect other tissues in the body?

No. DOMS only involves muscle and is not associated with damage to blood vessels, nerves, bones, joints, ligaments, or organs.

The disruption to muscle is not permanent and there is a rapid and complete resolution of discomfort. Recent research findings suggest that DOMS has an adaptive and protective role in the body as subsequent bouts of eccentric exercise produce much less soreness than previous bouts.
Appendix E: International Journal of Osteopathic Medicine – Guide for Authors

The journal Editors welcome contributions for publication from the following categories: Letters to the Editor, Reviews and Original Articles, Commentaries and Clinical Practice case studies with educational value.

Online Submission

Submission to this journal proceeds totally online (http://ees.elsevier.com/ijom) you will be guided stepwise through the creation and uploading of the various files. The system automatically converts source files to a single Adobe Acrobat PDF version of the article, which is used in the peer-review process. Please note that even though manuscript source files are converted to PDF at submission for the review process, these source files are needed for further processing after acceptance. All correspondence, including notification of the Editor's decision and requests for revision, takes place by e-mail and via the Author's homepage, removing the need for a hard-copy paper trail.

The above represents a very brief outline of this form of submission. It can be advantageous to print this "Guide for Authors" section from the site for reference in the subsequent stages of article preparation.

Submission of an article implies that the work described has not been published previously (except in the form of an abstract or as part of a published lecture or academic thesis), that it is not under consideration for publication elsewhere, that its publication is approved by all authors and tacitly or explicitly by the responsible authorities where the work was carried out, and that, if accepted, it will not be published elsewhere in the same form, in English or in any other language, without the written consent of the Publisher.

Types of contributions

Letters to the Editor As is common in biomedical journals the editorial board welcomes critical response to any aspect of the journal. In particular, letters that point out deficiencies and that add to, or further clarify points made in a recently published work, are welcomed. The Editorial Board reserves the right to offer authors the right of rebuttal, which may be published alongside the letter.

Reviews and Original Articles These should be either i) reports of new findings related to osteopathic medicine that are supported by research evidence. These should be original, previously unpublished works. The report will normally be divided into the following sections: abstract, introduction, materials and methods, results, discussion, conclusion, references. Or ii) critical or systematic review that seeks to summarise or draw conclusions from the established literature on a topic relevant to osteopathic medicine.

Short review The drawing together of present knowledge in a subject area, in order to provide a background for the reader not currently versed in the literature of a particular topic. Shorter in length than and not intended to be as comprehensive as that of the literature review paper. With more emphasis on outlining areas of deficit in the current literature that warrant further investigation.

Research Note Findings of interest arising from a larger study but not the primary aim of the research endeavour, for example short experiments aimed at establishing the reliability of new equipment used in the primary experiment or other incidental findings of interest, arising from, but not the topic of the primary research. Including further clarification of an experimental protocol after addition of further controls, or statistical reassessment of raw data.

Preliminary Findings Presentation of results from pilot studies which may establish a solid basis for further investigations. Format similar to original research report but with more emphasis in discussion of future studies and hypotheses arising from pilot study.
Commentaries Include articles that do not fit into the above criteria as original research. Includes commentary and essays especially in regards to history, philosophy, professional, educational, clinical, ethical, political and legal aspects of osteopathic medicine.

Clinical Practice Authors are encouraged to submit papers in one of the following formats: Case Report, Case Problem, and Evidence in Practice.

Case Reports usually document the management of one patient, with an emphasis on presentations that are unusual, rare or where there was an unexpected response to treatment e.g. an unexpected side effect or adverse reaction. Authors may also wish to present a case series where multiple occurrences of a similar phenomenon are documented. Preference will be given to reports that are prospective in their planning and utilise Single System Designs, including objective measures.

The aim of the Case Problem is to provide a more thorough discussion of the differential diagnosis of a clinical problem. The emphasis is on the clinical reasoning and logic employed in the diagnostic process.

The purpose of the Evidence in Practice report is to provide an account of the application of the recognised Evidence Based Medicine process to a real clinical problem. The paper should be written with reference to each of the following five steps: 1. Developing an answerable clinical question. 2. The processes employed in searching the literature for evidence. 3. The appraisal of evidence for usefulness and applicability. 4. Integrating the critical appraisal with existing clinical expertise and with the patient's unique biology, values, and circumstances. 5. Reflect on the process (steps 1-4), evaluating effectiveness, and identifying deficiencies.

Presentation of Typescripts

Your article should be typed on A4 paper, double-spaced with margins of at least 3cm. Number all pages consecutively beginning with the title page.

To facilitate anonymity, the author's names and any reference to their addresses should only appear on the title page. Please check your typescript carefully before you send it off, both for correct content and typographic errors. It is not possible to change the content of accepted typescripts during production.

Papers should be set out as follows, with each section beginning on a separate page:

Title page
To facilitate the peer-review process, two title pages are required. The first should carry just the title of the paper and no information that might identify the author or institution. The second should contain the following information: title of paper; full name(s) and address(es) of author(s) clearly indicating who is the corresponding author; you should give a maximum of four degrees/qualifications for each author and the current relevant appointment only; institutional affiliation; name, address, telephone, fax and e-mail of the corresponding author; source(s) of support in the form of funding and/or equipment.

Keywords
Include three to ten keywords. These should be indexing terms that may be published with the abstract with the aim of increasing the likely accessibility of your paper to potential readers searching the literature. Therefore, ensure keywords are descriptive of the study. Refer to http://www.nlm.nih.gov/mesh/meshhome.html for the MeSH thesaurus.

Abstract
Both qualitative and quantitative research approaches should be accompanied by a structured abstract. Commentaries and Essays may continue to use text based abstracts of no more than 150 words. All original articles should include the following headings in the abstract as appropriate: Background, Objective, Design, Setting, Methods, Subjects, Results, and Conclusions. As an absolute minimum: Objectives, Methods, Results, and Conclusions must be provided for all original articles. Abstracts for reviews of the literature (in particular systematic reviews and meta-analysis) should include the following headings as appropriate: Objectives, Data Sources, Study Selection, Data Extraction, Data Synthesis, Conclusions. Abstracts for Case Studies should include the following headings as appropriate: Background, Objectives, Clinical Features, Intervention and Outcomes, Conclusions.
Text
The text of observational and experimental articles is usually, but not necessarily, divided into sections with the headings; introduction, methods, results, results and discussion. In longer articles, headings should be used only to enhance the readability. Three categories of headings should be used:

- major ones should be typed in capital letter in the centre of the page and underlined
- secondary ones should be typed in lower case (with an initial capital letter) in the left hand margin and underlined
- minor ones typed in lower case and italicised

Do not use 'he', 'his' etc. here the sex of the person is unknown; say 'the patient' etc. Avoid inelegant alternatives such as 'he/she'. Avoid sexist language.

Statement of Competing Interests
When submitting a Research report you will need to consider if you, or any of your co-authors, are an Editor or Editorial Board member of the International Journal of Osteopathic Medicine. If this is the case you will need to include a section, at the end of your manuscript immediately before the reference section, called "Statement of Competing Interests". Example statement, which may require editing, is as follows: {Name of author} is an Editor of the Int J Osteopath Med; {Name of author} is a member of the Editorial Board of the Int J Osteopath Med but was not involved in review or editorial decisions regarding this manuscript.

References
Responsibility for the accuracy of bibliographic citations lies entirely with the Authors.

Citations in the text: Please ensure that every reference cited in the text is also present in the reference list (and vice versa). Avoid using references in the abstract. Unpublished results and personal communications are not recommended in the reference list, but may be mentioned in the text. If these references are included in the reference list they should follow the standard reference style of the journal and should include a substitution of the publication date with either "Unpublished results" or "Personal communication" Citation of a reference as "in press" implies that the item has been accepted for publication.

Text: Indicate references by superscript numbers in the text. The actual Authors can be referred to, but the reference number(s) must always be given.

List: Number the references in the list in the order in which they appear in the text.

Examples:

Reference to a journal publication:


Reference to a book:


Reference to a chapter in an edited book:


Note shortened form for last page number. e.g., 51-9, and that for more than 6 Authors the first 6 should be listed followed by "et al." For further details you are referred to "Uniform Requirements for Manuscripts submitted to Biomedical Journals" (J Am Med Assoc 1997;277:927-934) (see also http://www.nejm.org/general/text/requirements/1.htm)
Citing and listing of Web references. As a minimum, the full URL should be given. Any further information, if known (Author names, dates, reference to a source publication, etc.), should also be given. Web references can be listed separately (e.g., after the reference list) under a different heading if desired, or can be included in the reference list.

Tables, Illustrations and Figures
A detailed guide on electronic artwork is available on our website: http://www.elsevier.com/authors

Preparation of supplementary data. Elsevier now accepts electronic supplementary material (e-components) to support and enhance your scientific research. Supplementary files offer the Author additional possibilities to publish supporting applications, movies, animation sequences, high-resolution images, background datasets, sound clips and more. Supplementary files supplied will be published online alongside the electronic version of your article in Elsevier Web products, including ScienceDirect: http://www.sciencedirect.com. In order to ensure that your submitted material is directly usable, please ensure that data is provided in one of our recommended file formats. Authors should submit the material in electronic format together with the article and supply a concise and descriptive caption for each file. For more detailed instructions please visit our artwork instruction pages at http://www.elsevier.com/authors.

Illustrations and tables that have appeared elsewhere must be accompanied by written permission to reproduce them from the original publishers. This is necessary even if you are an author of the borrowed material. Borrowed material should be acknowledged in the captions in the exact wording required by the copyright holder. If not specified, use this style: 'Reproduced by kind permission of . . . (publishers) from . . . (reference).' Identifiable clinical photographs must be accompanied by written permission from the patient.

The text of original research for a quantitative or qualitative study is typically subdivided into the following sections:

Introduction
State the purpose of the article. Summarise the rationale for the study or observation. Give only strictly pertinent references and do not review the subject extensively. Do not include data or conclusions from the work being reported.

Materials and Methods
Describe your selection of observational or experimental subjects (including controls). Identify the methods, apparatus (manufacturer's name and address in parenthesis) and procedures in sufficient detail to allow workers to reproduce the results. Give references and brief descriptions for methods that have been published but are not well known; describe new methods and evaluate limitations.

Indicate whether procedures followed were in accordance with the ethical standards of the institution or regional committee responsible for ethical standards. Do not use patient names or initials. Take care to mask the identity of any subjects in illustrative material.

Results
Present results in logical sequence in the text, tables and illustrations. Do not repeat in the text all the data in the tables or illustrations. Emphasise or summarise only important observations.

Discussion
Emphasise the new and important aspects of the study and the conclusions that follow from them. Do not repeat in detail data or other material given in the introduction or the results section. Include implications of the findings and their limitations, include implications for future research. Relate the observations to other relevant studies. Link the conclusion with the goals of the study, but avoid unqualified statements and conclusions not completely supported by your data. State new hypothesis when warranted, but clearly label them as such. Recommendations, when appropriate, may be included.
Acknowledgments
In the appendix one or more statements should specify (a) contributions that need acknowledging, but do not justify authorship (b) acknowledgments of technical support (c) acknowledgments of financial and material support, specifying the nature of the support. Persons named in this section must have given their permission to be named. Authors are responsible for obtaining written permission from those acknowledged by name since readers may infer their endorsement of the data and conclusions.

IJOM Author Contribution Statement
All manuscripts submitted to the journal should be accompanied by an Author Contribution Statement. The purpose of the Statement is to give appropriate credit to each author for their role in the study. All persons listed as authors should have made substantive intellectual contributions to the research. To qualify for authorship each person listed should have made contributions in each of the following:
1) Contributions to conception and design; data acquisition; data analysis and interpretation;
2) Drafting of manuscript, or critical revision for important intellectual content;
3) All authors must have given approval to the final version of the manuscript submitted for consideration to publish.
Acquisition of funding; provision of resources; data collection; or general supervision, alone, is not sufficient justification for authorship. Contributors who do not meet the criteria for authorship as outlined above should be listed in the Acknowledgements section. Acknowledgements may include contributions of technical assistance, proof reading and editing, or assistance with resources and funding. The statement may be published in the paper as appropriate.
Example of suggested format. Note the use of author initials.
AB conceived the idea for the study. AB and CD contributed to the design and planning of the research. All authors were involved in data collection. AB and EF analysed the data. AB and CD wrote the first draft of the manuscript. EF coordinated funding for the project. All authors edited and approved the final version of the manuscript.

Funding body agreements and policies
Elsevier has established agreements and developed policies to allow authors whose articles appear in journals published by Elsevier, to comply with potential manuscript archiving requirements as specified as conditions of their grant awards. To learn more about existing agreements and policies please visit http://www.elsevier.com/fundingbodies

Copyright
Upon acceptance of an article, authors will be asked to sign a "Journal Publishing Agreement" (for more information on this and copyright see http://www.elsevier.com/copyright. Acceptance of the agreement will ensure the widest possible dissemination of information. An e-mail (or letter) will be sent to the corresponding author confirming receipt of the manuscript together with a 'Journal Publishing Agreement' form or a link to the online version of this agreement. If excerpts from other copyrighted works are included, the author(s) must obtain written permission from the copyright owners and credit the source(s) in the article. Elsevier has preprinted forms for use by authors in these cases: contact Elsevier's Rights Department, Philadelphia, PA, USA: phone (+1) 215 239 3804, fax (+1) 215 239 3805, healthpermissions@elsevier.com. Requests may also be completed online via the Elsevier homepage http://www.elsevier.com/locate/permissions.

Page Proofs
One set of page proofs will be sent by e-mail to the corresponding author (if we do not have an e-mail address then paper proofs will be sent by post). Elsevier now sends PDF proofs which can be annotated, for this you will need to download Adobe Reader version 7 (or higher) available free from http://www.adobe.com/products/acrobat/readstep2.html. Instructions on how to annotate PDF files will accompany the proofs. The exact system requirements are given at the Adobe site: http://www.adobe.com/products/acrobat/acrsystemreqs.html#70win.

If you do not wish to use the PDF annotations function, you may list the corrections (including replies to the Query Form) and return to Elsevier in an e-mail. Please list your corrections quoting line number. If, for any reason, this is not possible, then mark the corrections and any other comments (including replies to the Query Form) on a printout of your proof and return by fax, or scan the pages and e-mail, or by post.
Please use this proof only for checking the typesetting, editing, completeness and correctness of the text, tables and figures. Significant changes to the article as accepted for publication will only be considered at this stage with permission from the Editor. We will do everything possible to get your article published quickly and accurately. Therefore, it is important to ensure that all of your corrections are sent back to us in one communication, please check carefully before replying, as inclusion of any subsequent corrections cannot be guaranteed. Proofreading is solely your responsibility. Note that Elsevier may proceed with the publication of your article if no response is received.

Author Enquiries
For enquiries relating to the submission of articles (including electronic submission where available) please visit this journal's homepage at http://www.elsevier.com/locate/ijosm. You can track accepted articles at http://www.elsevier.com(trackarticle) and set up e-mail alerts to inform you of when an article's status has changed. Also accessible from here is information on copyright, frequently asked questions and more.

Contact details for questions arising after acceptance of an article, especially those relating to proofs, will be provided by the publisher.

Language services. Authors who require information about language editing and copyediting services pre- and post-submission please visit http://www.elsevier.com/wps/find/authorshome.authorslocate/languagepolishing or contact authorsupport@elsevier.com for more information. Please note Elsevier neither endorses nor takes responsibility for any products, goods or services offered by outside vendors through our services or in any advertising. For more information please refer to our Terms and Conditions http://www.elsevier.com/termsandconditions.

Offprints
The corresponding author, at no cost, will be provided with a PDF file of the article via e-mail or, alternatively, 25 free paper offprints. The PDF file is a watermarked version of the published article and includes a cover sheet with the journal cover image and a disclaimer outlining the terms and conditions of use. Additional paper offprints can be ordered by the authors. An order form with prices will be sent to the corresponding author.

Checklist
Please check your typescript carefully before you send it off to the Editorial Office, both for correct content and typographical errors, as it is not possible to change the content of accepted typescripts during the production process.

• One copy of typescript and illustrations
• Reference list in correct style
• Written Permission from original publishers and authors to reproduce any borrowed any borrowed material