THE EFFECTS OF HYPOCAPNIA ON CARDIAC ELECTRICAL ACTIVITY AND HEART FUNCTION AND ITS RELAVENCE TO THE DIAGNOSIS OF CORONARY ARTERY DISEASE

by

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Abstract

Current methods used in the diagnosis of coronary artery disease vary in sensitivity and specificity and have a number of limitations. The aim of this thesis investigation was to explore a new technique for inducing hypocapnia in resting subjects and investigate whether this technique has any clinical applications in the diagnosis of coronary artery disease.

In 18 healthy subjects, the effects of hypocapnia, induced by mechanical hyperventilation (in 21% or 15% inspired O_2), on cardiac electrical activity and heart function were investigated using an electrocardiogram (ECG) and echocardiogram. In addition, a pilot study was conducted to examine the effect of hypocapnia on the ECG of four patients suffering from coronary artery disease with stable angina.

Experiments using mechanical hyperventilation showed that the most severe hypocapnia tolerable ($PetCO_2$ = 20 ± 0mmHg) in normal healthy subjects causes a significant increase in T wave amplitude (increase of up to 0.09 ± 0.02mV, $P < 0.01$) in the anteroseptal leads (V_{1-3}) of 18 normal subjects but these changes do not exceed the clinical thresholds for hyperacute T wave amplitudes. Hypocapnia did not cause any other significant ECG or echocardiographic changes during mechanical hyperventilation.

Reducing inspired O_2 to 15% during hypocapnia in nine normal subjects did not accentuate any of the T wave changes seen during hypocapnia, nor did it cause any clinically significant changes to appear.

In two patients suffering from coronary artery disease with stable angina, no clinically significant ECG changes were seen during hypocapnia. These patients were taking isosorbide mononitrate medication which could have interfered with the vasoconstrictive effects of hypocapnia. In two patients not taking this type of medication, small increases in T wave amplitude (of up to 0.05 ± 0.01mV) and decreases in ST segment height (of up to 0.05 ± 0.01mV) were observed.

These results show that hypocapnia, induced by mechanical hyperventilation, of the greatest severity tolerable in normal subjects, does not induce clinically significant ECG changes in normal healthy subjects as has been previously suggested. Preliminary results from four patients suffering from stable angina suggest that hypocapnia does cause small ECG changes but these are not consistent and are unlikely to be of clinical importance. However, conclusions about the clinical applications of this technique cannot be made until more patients are studied.

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Chapter 1

Introduction

The aim of this thesis is to investigate the effects of hypocapnia induced by mechanical hyperventilation on cardiac electrical activity and heart function. This is of interest because of the potential clinical applications of this technique in the diagnosis of coronary artery disease.

In patients who suffer from coronary artery disease, a narrowing of the coronary artery diameter (due to a build up of atherosclerotic plaques) reduces blood flow and O_2 delivery to the myocardium (Braunwald *et al.*, 2001). If this occlusion of the coronary artery is of sufficient severity, myocardial ischemia will develop because of an imbalance between the O_2 supply and the O_2 demand. This causes abnormalities in contractility and electrical activity within the heart which can be detected using a variety of cardiac imaging techniques.

For patients who experience chest pain (angina) during physical activity, the presence of coronary artery disease can be detected using invasive anatomical testing for obstructive coronary artery disease (coronary angiography) or non-invasive functional testing for myocardial ischemia (Cooper *et al.*, 2010). The current clinical procedures for diagnosing coronary artery disease with stable angina are discussed below. All previous literature described in this thesis refers to experiments carried out in unanaesthetised man unless otherwise stated.

1.1. Diagnostic tests for coronary artery disease

According to recent guidelines published by the National Institute for Clinical Excellence (NICE) (Cooper *et al.*, 2010), patients who present with unknown chest pain should undergo a clinical risk assessment to establish the likelihood that their chest pain is of cardiac origin. During this risk assessment, clinical history and risk factors for cardiovascular disease are recorded and a physical examination takes place. The decision to recommend further diagnostic testing for coronary artery disease is based on the outcome of the clinical risk assessment. According to the guidelines, patients with a high risk of having coronary artery disease (60-90% risk) should be recommended for invasive anatomical testing with coronary angiography (see chapter 1.1.1). Those with a moderate risk of developing coronary artery disease (30-60% risk) should undergo functional testing for myocardial ischemia (see chapter 1.1.2-1.1.5). The methods, limitations and clinical sensitivity and specificity for coronary artery disease of these functional tests are described below. The sensitivity of a diagnostic test refers to the percentage of cases in which the test positively identifies the existence of disease. The specificity of a diagnostic test refers to the percentage of cases in which the test is negative in a patient without disease.

1.1.1. Coronary angiography

Coronary angiography is an invasive, anatomical test for coronary artery disease and is considered the reference 'gold' standard test to which all other diagnostic tests for coronary artery disease are compared (Pepine, 1992; Braunwald *et al.*, 2001). The technique uses a catheter inserted into the coronary artery, via the femoral/brachial/radial artery, into which a radiocontrast agent is injected and an x-ray image of the heart is recorded (Braunwald *et al.*, 2001). Flow of the radiocontrast agent through arteries that are partially occluded is restricted

and this can be identified on the radiograph. A coronary artery with an occlusion of ≥70% is considered severe and likely to cause angina (*i.e.* a positive result for coronary artery disease with stable angina) (Cooper *et al.*, 2010). This technique allows the identification of the specific artery affected by the coronary artery disease.

The main limitations of this technique are that it is highly invasive, expensive and involves exposing the patient to a significant dose of radiation (Cooper *et al.*, 2010). In addition, it has a relatively high threshold for detection (atherosclerotic plaques must be large [≥70% occlusion] before they are noticeable on an angiogram) and does not give information on the functional effects of reduced blood flow (*i.e.* identify the actual presence or absence of myocardial ischemia) (Pepine, 1992).

1.1.2. Exercise ECG

Electrocardiogram (ECG) exercise stress testing is a non-invasive, functional test for myocardial ischemia, commonly used in the diagnosis of coronary artery disease. The sensitivity, specificity and limitations of the test are summarised in table 1.1.

In patients with suspected coronary artery disease, the physiological stress induced by exercise increases the workload of the heart, therefore augmenting O_2 demand and creating an O_2 deficit. This happens because the coronary blood supply cannot match the demand due to obstructions in the diseased arteries (Pepine, 1992). This causes changes in cardiac electrical activity which can be identified on an ECG waveform.

The original exercise stress test was developed by Master *et al.,* (1942) and involved a double

two-step test of varying intensity, adjusted for each patient according to their exercise tolerance. ECGs were recorded during the test and revealed ST elevation or depression of >0.5mm or flattening/inversion of the T wave in 39% of 54 patients with normal resting ECGs and 67% in 29 patients with abnormal resting ECGs indicating the existence of coronary artery disease.

Table 1.1. Sensitivity and Specificity of current diagnostic tests for coronary artery disease.

Diagnostic Technique	Sensitivity	Specificity	Limitations	Recommended by NICE?
Coronary Angiography	Gold standard	Gold standard	Highly invasive, expesive, exposure to radiation	Yes - in patients with 60-90% risk of developing CAD
Exercise ECG[1]	68%	77%	Lower accuracy than other techniques, little use in patients with limited exercise capacity	No - no longer considered cost effective by the NHS
MPS with SPECT[2]	88%	73%	Side effects of stress agents, exposure to radiation, requires two visits to the hospital	Yes - in patients with 30-60% risk of developing CAD
Stress echocardiography[2]	79%	87%	Side effects of stress agents, accuracy depends on the observer	Yes - in patients with 30-60% risk of developing CAD
Stress perfusion MRI[3]	91%	81%	Side effects of stress agents, access to MRI scanners, can cause claustrophobia	Yes - in patients with 30-60% risk of developing CAD
Stress wall motion MRI[3]	83%	86%	Side effects of stress agents, access to MRI scanners, can cause claustrophobia	Yes - in patients with 30-60% risk of developing CAD

Table 1.1. References: [1]Gianrossi, *et al.*, (1989), [2]Heijenbrok, *et al.*, (2007), [3]Nandalur, *et al.*, (2007).

Treadmill exercise testing commonly uses the Bruce protocol (Pepine, 1992). This multistage exercise test consists of four stages of exercise of increasing speed and gradient, each lasting 3 minutes with the final stage being carried out until exhaustion (Bruce *et al.*, 1963). Each

stage represents an increase in exercise intensity of three metabolic equivalents (3 times the resting metabolic rate) (Pepine, 1992). The test is stopped if patients experience chest pain or ischemic ECG changes occur.

The sensitivity and specificity of the ECG exercise stress test was assessed in a meta-analysis of 147 studies by Gianrossi *et al.*, 1989. All studies compared the standard ECG exercise test to coronary angiography (reference standard). The ECG exercise test was found to have a sensitivity of 68% and a specificity of 77% for coronary artery disease.

The ECG exercise test is considered safe if done under supervision and only induces myocardial infarction/death in 1/2500 patients tested (Gibbons *et al.*, 1997). Limitations of the technique include a low detection rate in patients with single vessel disease. It also has less use in patients who have a limited exercise capacity because a heart rate of >85% of maximal must be achieved for the test to be effective (Braunwald *et al.*, 2001). In addition, ECGs can be difficult to measure during the test because movement of the arms and legs can create muscle artefacts which distort the ECG waveform (Mason & Likar, 1966). This often makes ECG recordings illegible, although this problem can be avoided by using the modified ECG electrode placement devised by Mason & Likar (1966). There is some dispute as to whether this modified electrode placement alters the ECG waveform significantly from the standard ECG (Kleiner *et al.*, 1978; Takuma *et al.*, 1995; Jowett *et al.*, 2005; Rautaharju *et al.*, 1980; Pahlm *et al.*, 1992; Krucoff *et al.*, 1994; Gamble *et al.*, 1984; Papouchado *et al.*, 1987; Edenbrandt *et al.*, 1989; Sevilla *et al.*, 1989).

Due to its low sensitivity and specificity compared to other diagnostic techniques (Gianrossi

et al., 1989; table 1.1), the ECG exercise test is no longer considered cost effective for diagnosing coronary artery disease and therefore is not recommended for use by NICE guidelines (Cooper *et al.*, 2010).

1.1.3. MPS with SPECT

Myocardial perfusion scintigraphy (MPS) using single proton emission computed tomography (SPECT) is a non-invasive functional test for myocardial ischemia. The sensitivity, specificity and main limitations of the test are summarised in table 1.1.

This technique uses radiopharmaceutical tracers (thallium TI-201 or technetium Tc-99m) which are injected into the coronary arteries at rest and during myocardial stress (exercise or pharmacological) (Cooper *et al.*, 2010). The distribution of the tracer within the myocardium reflects regional blood flow and is measured using SPECT imaging. In patients with coronary artery disease (with stable angina), this blood flow is reduced during myocardial stress indicating the presence of myocardial ischemia. Stress can be induced by exercise or pharmacologically using stress agents such as dobutamine (increases heart rate and myocardial contractility), dipyridamole or adenosine (increases vasodilatation in healthy coronary arteries causing blood to flow away from diseased arteries) (Heijenbrok-Kal *et al.*, 2007).

When coronary angiography is use as the reference standard, the sensitivity and specificity of MPS with SPECT varies depending on the techniques or pharmacological agent used to induce myocardial stress. Used with exercise, the test has a sensitivity of 88% and specificity of 67% (Heijenbrok-Kal *et al.*, 2007). When pharmacological stress agents are used to induce

myocardial stress, MPS with SPECT has a sensitivity of 91% (adenosine), 90% (dipyridamole) or 84% (dobutamine) and a specificity of 81% (adenosine), 75% (dipyridamole) or 73% (dobutamine). The overall sensitivity and specificity of MPS with SPECT for coronary artery disease is 88% and 73% respectively (Heijenbrok-Kal *et al.*, 2007).

An advantage of MPS with SPECT is the openness of the scanning equipment which, unlike MRI, is unlikely to cause claustrophobia. In addition, it can be used in obese patients where alternative imaging techniques (such as ECG and echocardiography) are impractical (Cooper *et al.*, 2010). The disadvantages of the technique are that it involves a significant dose of radiation and two separate visits to the hospital. On occasions where pharmacological stress agents are used, side effects such as shortness of breath, headaches, dizziness, nausea, flushing, arrhythmias, airway obstruction or acute bronchospasm can occur (Cooper *et al.*, 2010).

1.1.4. Stress echocardiography

Stress echocardiography is a functional test for myocardial ischemia in which ultrasound is used to image the left ventricle and detect changes in wall motion and thickening during myocardial stress. The technique avoids the exposure to radiation used in alternative tests and can be performed using equipment that is widely available in a healthcare setting. Additional advantages of this technique are that it is low cost (in comparison to MPS with SPECT, MRI or coronary angiography), short in duration and allows identification of co-existing cardiac diseases (Braunwald *et al.*, 2001). It is therefore recommended for use in patients with a 30-60% risk of having coronary artery disease with stable angina by NICE guidelines (Cooper *et*

al., 2010).

Stress is induced either by exercise or pharmacologically using stress agents such as dobutamine, dipyridamole or adenosine (Heijenbrok-Kal *et al.,* 2007). Echocardiography is used at rest and then during stress to find evidence of decreased wall motion/thickening in at least one left ventricular segment and/or compensatory hyperkinesis in a non-ischemic segment (Cooper *et al.*, 2010).

As with MPS with SPECT, the sensitivity and specificity of stress echocardiography for coronary artery disease varies depending on the technique or pharmacological stress agent used to induce myocardial stress. A meta-analysis by Heijenbrok-Kal *et al.,* (2007) found that when compared to coronary angiography as the reference standard, the sensitivity of stress echocardiography is highest with exercise stress (83%) compared with adenosine (79%), dipyridamole (72%) or dobutamine (81%). Conversely, exercise stress echocardiography was found to have the lowest specificity (84%) when compared to the pharmacological stress agents, adenosine (92%), dipyridamole (95%) or dobutamine (84%). According to Heijenbrok-Kal *et al.,* (2007) the overall sensitivity and specificity of stress echocardiography for coronary artery disease is 79% and 87% respectively (table 1.1).

The disadvantages of stress echocardiography centre on the difficulty in gaining accurate images of the left ventricle in some patients and a high dependence on the echocardiography technician's imaging skills and experience for accurate diagnosis (Braunwald *et al.*, 2001; Geleijnse *et al.,* 1995; Picano *et al.,* 1991). For example, stress echocardiography is not appropriate for use in patients with obesity, chronic obstructive airways disease or chest

deformity where an appropriate acoustic window cannot be obtained (Cooper *et al.*, 2010). In addition, the use of pharmacological stress agents can result in unpleasant side effects (see section 1.1.3, page 7).

1.1.5. Stress MRI

Stress magnetic resonance imaging (MRI) is a non-invasive functional test for myocardial ischemia. MRI uses a powerful magnetic field and radio frequency pulses to produce detailed pictures of the myocardium with high contrast and excellent spatial resolution (Cooper *et al.*, 2010). These images can be examined on a computer and are used to assess both myocardial perfusion defects and wall motion abnormalities. The sensitivity, specificity and main limitations of both perfusion and wall motion imaging are summarised in table 1.1.

Stress perfusion MRI assesses myocardial perfusion defects induced by the same pharmacological stress agents used in stress echocardiography and MPS with SPECT (Nandalur *et al.*, 2007). Stress wall motion MRI images regional wall motion and thickening at rest and during pharmacological stress. If myocardial ischemia is present, wall motion and thickening will become inhibited.

Nandalur *et al.*, (2007) assessed the sensitivity and specificity of stress perfusion and wall motion MRI for coronary artery disease. Both techniques were compared to coronary angiography as a reference standard in a total of 37 studies. Stress perfusion MRI was found to have a sensitivity and specificity of 91% and 81% respectively. Stress wall motion MRI was estimated to have a sensitivity of 83% and specificity of 86%. The sensitivity of stress wall motion MRI was improved to 85% when only studies utilizing exercise or dobutamine

were included in the analysis.

The main advantages of stress MRI are that it is non-invasive, does not involve exposure to radiation and has a high sensitivity and specificity for coronary artery disease when compared to other functional tests for myocardial ischemia (table 1.1). The primary disadvantages to both stress MRI techniques are that they are not appropriate for patients with ferromagnetic intercranial surgical clips, metallic intraocular foreign bodies, pace makers or in those who are morbidly obese where accurate images cannot be obtained (Cooper *et al.,* 2010). In addition, access to MRI scanners can sometimes be limited, patients who suffer from claustrophobia may decline to be tested and the pharmacological stress agents used can cause side effects (see section 1.1.3, page 7).

1.1.6. Summary of diagnostic tests for coronary artery disease

All previously described anatomical and functional tests for myocardial ischemia are established diagnostic tests for coronary artery disease. In the UK, the decision as to which tests are used in clinical practice is based on the recommendations of the National Institute for Clinical Excellence. These recommendations are based on the accuracy of the test, the cost effectiveness of the test and the advantages and disadvantages to the patient. In recently published guidelines on the diagnosis of coronary artery disease with angina (Cooper *et al.,* 2010), coronary angiography, MPS with SPECT, stress echocardiography and stress MRI were all recommended for use in clinical practice. Despite its wide use in clinical practice, the exercise ECG test is no longer recommended due to its low sensitivity and specificity compared with other functional tests. It should be noted that all the aforementioned tests have limitations, such as exposure to radiation, side effects of stress agents or expense and

accessibility to equipment. Therefore, the advent of any new diagnostic test is welcomed, provided it delivers the right balance of accuracy to cost benefit and does not place the patient at any unnecessary risk.

This thesis is concerned with a novel method of inducing hypocapnia using mechanical hyperventilation. Below, the technique is described, along with the physiological effects of hypocapnia and the potential applications of this technique in the diagnosis of coronary artery disease. The experiments within this thesis investigation were designed to develop this technique with its potential clinical applications in mind. Therefore, all experiments have been conducted in normal healthy subjects prior to recruiting patients with established coronary artery disease.

1.2. Hypocapnia

In healthy humans at rest, the partial pressure of carbon dioxide in arterial blood ($PaCO_2$) is between 35-45mmHg (Ganong, 1997). Therefore, hypocapnia is defined as an abnormally low tension of CO_2 (<35mmHg) in arterial blood (Steadman, 1972). A $PaCO_2$ of 34mmHg represents a very mild form of hypocapnia. In the experiments presented in this thesis, hypocapnia was defined as the lowest $PaCO_2$ possible, without inducing tetany and paresthesia which are known to occur below 20mmHg (Macefield & Burke, 1991). Hypocapnia is caused by an increase in ventilation beyond what is needed to maintain blood gases within a normal range (hyperventilation) (Steadman, 1972). Hyperventilation therefore causes a greater amount of CO_2 to be exhaled than is produced in the body. Hyperventilation can be induced voluntarily or by mechanical ventilation.

1.2.1. Physiological effects of hypocapnia

Hypocapnia decreases the concentration of H^+ and HCO_3^- in the blood due to the bicarbonate buffering system (Klocke, 1987) (Equation 1.1) causing pH to increase. This increased arterial blood pH and the direct effect of decreased CO_2 on haemoglobin causes a leftward shift of the oxygen dissociation curve (figure 1.1) (Roughton, 1964). This increased affinity of haemoglobin for O_2 makes it more difficult for oxygen to dissociate into the tissues.

Equation 1.1. Bicarbonate buffering system

$$CO_2 + H_2O \Leftrightarrow H_2CO_3 \Leftrightarrow H^+ + HCO_3^-$$

Hypocapnia (15-22mmHg) also causes tetany and paraesthesia (Barker *et al.*, 1939; Edmondson *et al.*, 1975; Kruyswijk *et al.*, 1986; Macefield & Burke, 1991; McCance, 1932; Miyagi *et al.*, 1989; Neill & Hattenhauer, 1975; Previtali *et al.*, 1989; Scherf & Schlachman, 1947; Thompson, 1943; Yasue *et al.*, 1978; Yu *et al.*, 1959) thought to be caused be a decrease in arterial Ca^{2+} (Edmondson *et al.*, 1975). Hypocapnia (17-32mmHg) induces a decrease of 20-39% in specific airway conductance (Sterling, 1968; Jamison *et al.*, 1987) due to bronchonstriction (indicated by an 13% increase in total respiratory resistance) (Ocain *et al.*, 1979). Hypocapnia (18-29mmHg) also causes a reduction of 33-81% in cerebral blood flow via cerebral vasoconstriction (Kety & Schmidt, 1946; Weckesser *et al.*, 1999; Yokoyama *et al.*, 2008) and decreases respiratory sinus arrhythmia by 33-74% caused by an attenuation in the central respiratory rhythm (Cooper *et al.*, 2004; Sasano *et al.*, 2002). The affects of hypocapnia on muscular function, airway conductance, cerebral blood flow and central respiratory rhythm are not relevant to this thesis investigation and therefore do not require further discussion.

Figure 1.1. The oxygen dissociation curve shifts to the left at high pH (7.6 [$PaCO_2$ = 20mmHg])

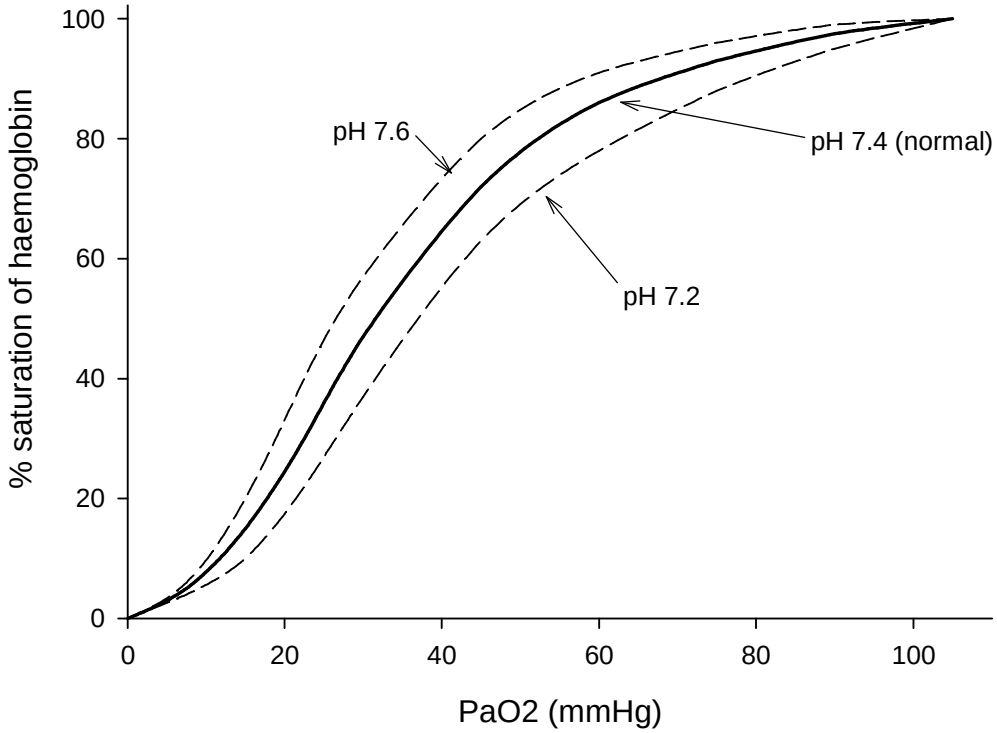


Figure 1.1. Oxygen dissociation curve at different levels of blood pH. A pH of 7.6 corresponds to a decrease in $PaCO_2$ (hypocapnia). From Roughton, F. (1964). Transport of oxygen and carbon dioxide. In *Handbook of Physiology – Section 3: Respiration (Volume 1)*, eds. Fenn, W. & Rahn, H., pp. 767-825. American Physiological Society, Washington, D.C.

Studies conducted in both animal models and humans suggest that hypocapnia causes a reduction in coronary blood flow via coronary vasoconstriction. For example, hypocapnia (23mmHg) causes a reduction in myocardial blood flow by up to 50% in anaesthetized pigs (measured by the thermodilution technique) (Karlsson *et al.*, 1994) and a reduction coronary blood flow of 23-33% in anaesthetized dogs (measured by electromagnetic and xenon clearance techniques) (Vance *et al.*, 1973; Coetzee *et al.*, 1984). In normal healthy humans, measurement of coronary blood flow (measured by the nitrous oxide technique and positron emission tomography) have revealed that hypocapnia (19-20 mmHg) reduces overall coronary blood flow by 30% (Rowe *et al.*, 1962; Yokoyama *et al.*, 2008).

In patients with coronary artery disease, coronary blood flow has been shown to increase by 13% (Wilson *et al.*, 1981), decrease by 12% (Neill & Hattenhauer, 1975) and not change at all (Kazmaier *et al.*, 1998). The variability in responses can be explained by the severity of hypocapnia induced. Both Wilson *et al.,* (1981) and Neill & Hattenhauer (1975) used voluntary hyperventilation to induce hypocapnia. Neill & Hattenhauer (1975) demonstrated a decrease in $PaCO_2$ to 19mmHg and this coincided with a decrease 12% in coronary blood flow. Wilson *et al.*, (1981) demonstrated an increase in coronary blood flow but did not measure $PaCO_2$ or $PetCO_2$ and patients in this study were only hyperventilated for a maximum of 6 minutes. It is therefore possible that patients were not sufficiently hypocapnic to cause coronary vasoconstriction. For the same reason, the findings of the Kazmaier *et al.,* (1998) study should be taken with caution. Although patients were mechanically hyperventilated, it seems the lack of changes in coronary blood flow may have been due to the fact that $PaCO_2$ only fell to 31mmHg and therefore may not have been severe enough to stimulate coronary vasoconstriction.

The reason for and causes of hypocapnia induced coronary vasoconstriction are unknown. In the pulmonary vasculature, decreases in $PetCO_2$ result in pulmonary artery vasodilatation which increases pulmonary blood flow (Balanos *et al.*, 2003; Dorrington *et al.*, 2010). The reason for this increase pulmonary blood flow is that hypocapnia is caused by hyperventilation which also increases O_2 availability in the alveoli. In order to maintain ventilation-perfusion matching, pulmonary vasodilatation must occur to enable increased blood flow, allowing greater gaseous exchange at the lungs. In this case, there is an obvious reason for hypocapnia induced pulmonary vasodilatation. There is on the other hand, no clear advantage to reducing coronary blood flow to the myocardium during hyperventilation. This

makes it difficult to postulate the mechanism which might cause coronary vasoconstriction. Such mechanisms are speculated below;

Hypocapnia is known to cause vasoconstriction in cerebral vascular bed (Kety & Schmidt, 1946; Weckesser *et al.*, 1999; Yokoyama *et al.*, 2008). To explain this phenomenon, it has been suggested that the decrease in $PaCO_2$ and increase in arterial blood pH that occurs during hypocapnia might cause an increase in intracellular Ca^{2+} in the vascular smooth muscle via stimulation of a pathway such as inositol 1,4,5-trisphosphate (Mirro *et al.*, 1992). It is thought that this increase in intracellular Ca^{2+} may cause contraction of the vascular smooth muscle resulting in cerebral vasoconstriction. It is possible that a similar mechanism exists in the coronary vascular bed causing hypocapnia induced coronary vasoconstriction.

An alternative possible mechanism to explain the reduction in coronary blood flow observed during hypocapnia is the availability of the vasodilator, nitric oxide in the coronary vasculature. Nitric oxide is a well known regulator of vascular resistance in the coronary circulation and its production in the endothelium of the coronary arteries is thought to maintain resting coronary artery diameter (Quyyumi *et al.*, 1995; Lefroy *et al.*, 1993). Inhibition of nitric oxide production, with substances such as N^G-monomethyl-L-arginine (L-NMMA) have been shown to reduce coronary artery diameter by up to 13% and increase coronary vascular resistance by up to 22% (Quyyumi *et al.*, 1995; Lefroy *et al.*, 1993). It can therefore be speculated that hypocapnia causes an increase in L-NMMA in the endothelium of the coronary arteries by stimulation of an (unknown) pathway. This would reduce nitric oxide production, inhibiting baseline coronary artery vasodilatation, causing increased coronary vascular resistance and decreased coronary blood flow.

It is suspected that coronary vasoconstriction induced by hypocapnia coupled with reduced oxygen dissociation into the myocardial tissues causes myocardial ischemia (Laffey & Kavanagh, 2002; Neill & Hattenhauer, 1975; Rutherford *et al.*, 2005). If this is the case, hypocapnia may be a useful alternative type of myocardial stress to be utilised in non-invasive functional diagnostic testing for coronary artery disease with stable angina. One advantage of using hypocapnia over exercise stress to induce myocardial stress is that it does not require any physical exertion and therefore would be appropriate for use in patients with limited exercise capacity. In addition, inducing hypocapnia may be more preferable than using pharmacological stress agents because it has no known side effects and can be accurately controlled throughout the test procedure. Other advantages to using hypocapnia as a form of myocardial stress are that it utilises equipment that is commonly available in a clinical setting (mechanical ventilators), is quick to perform (takes no longer than an hour to perform as a test) and therefore could be considered as cost effective, provided that it is found to have a high sensitivity and specificity for coronary artery disease.

It was the aim of this thesis investigation to extent what is already known about the effects of hypocapnia on cardiac electrical activity and heart function, measured by established techniques such as the ECG and echocardiogram. Previous studies have investigated the effects of hypocapnia, induced by voluntary hyperventilation, on the ECG. However, the use of voluntary hyperventilation to induce hypocapnia is limited when studying its effects on the ECG (Rutherford *et al.*, 2005; see below, sections 1.2.2-1.2.3). This thesis investigation aims to eliminate these limitations using a novel method of mechanical hyperventilation to induce hypocapnia.

1.2.2. Voluntary hyperventilation vs. mechanical hyperventilation

Voluntary hyperventilation is most commonly used in studies examining the effects of hypocapnia. However, this technique presents a number of limitations. Primarily, it is difficult to induce a consistent level of hypocapnia during voluntary hyperventilation because tidal volume and breathing frequency cannot be controlled. This issue can be resolved by sampling $PetCO_2$ instantaneously and automatically adjusting the concentration of CO_2 in inspired air to compensate for changes in breathing (Balanos *et al.*, 2003; Talbot *et al.*, 2008; Dorrington *et al.*, 2010). However, even if this method is utilised, inconsistent inflation volumes still limit the use of voluntary hyperventilation in studies using the ECG because alterations in inflation volume can cause changes in ECG wave amplitudes, independently of the physiological effects of hypocapnia (Rutherford *et al.*, 2005). An increase in chest inflation (of up to 0.6L) causes an increase in the distance of the ECG electrodes from the heart, causing a decrease in ECG wave amplitudes (R and T waves) of up to 0.2-0.3mV. Mechanical hyperventilation enables tidal volume and breathing frequency to be kept constant at a predefined level (Cooper *et al.*, 2004), which is advantageous because it enables a consistent and prolonged hypocapnia to be induced. This also eliminates the problems associated with inconsistent inflation volumes and the ECG (Rutherford *et al.*, 2005).

Voluntary hyperventilation itself, increases metabolic rate (through increased work of respiratory muscles) creating a paradox where CO_2 production is increased making hypocapnia more difficult to induce. Mechanical hyperventilation unloads the effort of the respiratory muscles, reducing metabolic rate, therefore enabling significant reductions in $PaCO_2$ with only small increases in tidal volume and breathing frequency. Therefore,

mechanical hyperventilation represents a more effective technique for inducing hypocapnia and studying its effects on physiological systems within the body (Cooper *et al.*, 2003; 2004).

1.2.3. Voluntary hyperventilation and the ECG

Several studies that utilise voluntary hyperventilation have suggested that hypocapnia causes ischemic ECG changes in both normal subjects and patients suffering from coronary artery disease.

Normal subjects

Table 1.2 shows that in normal subjects, hypocapnia (14-24mmHg) induced by voluntary hyperventilation causes;

- a decrease in the amplitude of the T wave by up to 0.4mV (lead II)
- ST segment elevation of up to 0.15mV (Leads II, III)
- QTc prolongation of up to 0.4 seconds

The effects of voluntary hyperventilation on T wave were not consistent. Changes were only evident in 8-57% of subjects and therefore cannot be considered reproducible (Barker *et al.*, 1939; Golden *et al.*, 1975; Joy & Trump, 1981; Kemp & Ellestad, 1968; Lary & Goldschlager, 1974; McCance, 1932; Wasserberger *et al.*, 1956). In addition, many studies did not measure end tidal CO_2 ($PetCO_2$) and so the degree of induced hypocapnia cannot be quantified (McCance, 1932; Thompson, 1943; Christensen, 1946; Scherf & Schlachman, 1947; Wasserberger *et al.*, 1956; Kemp & Ellestad, 1968; Biberman *et al.*, 1971; Lary & Goldschlager, 1974; Joy & Trump, 1981; Jacobs *et al.*, 1974).

Table 1.2 shows the lack of consistent changes in the T wave and ST segment during voluntary hyperventilation in normal subjects.

Author	Duration of hyperventilation	Hypocapnia ?	ECG lead	Mean T wave change	Mean ST segment changes	QTc interval	Notes
Christensen, 1946 (n = 3)	3-8 minutes	No data	I, II, III	Lead I: -0.2mV *** Lead II: -0.4mV *** Lead III: -0.16mV ***	Lead I: 0 mV Lead II: -0.15mV Lead III: -0.15mV	No data	Similar R wave changes
Barker *et al.*, 1939 (n = 7)	8-16 minutes	No data	I, II, III	Lead I: -0.13mV ** Lead II: -0.08mV * Lead III: +0.01mV ***	No data	+11ms *	Similar R wave changes, T wave changes only seen in 4/7 subjects (57%)
McCance, 1932 (n = 2)	No data	No data	I, II, III	Lead I: -0.35mV ** Lead II: -0.25mV ** Lead III: +0.1mV **	No data	No data	T wave changes were seen in 1/2 (50%) of subjects
Biberman *et al.*, 1971 (n = 12)	30-60 seconds	24 mmHg	II, V4	Lead II: -0.05mV *	No data	+40ms *	
Scherf & Schlachman, 1947 (n = 35)	5-15 minutes	No data	I, II, III	Lead I: -0.03mV* Lead II: -0.03mV* Lead III: +0.01mV*	No data	No data	Similar R wave changes
Golden *et al.*, 1975 (n = 27)	3 minutes	14 mmHg	I, II, III	Mean change: +0.004mV *	No data	No data	Changes of -0.2mV in 6/72 subjects (8%)
Joy & Trump, 1981 (n = 103)	15-30 seconds	No data	12 leads	No data	No data	No data	T wave/ST segement changes in 55/103 (53%) subjects
Kemp & Ellestad, 1968 (n = 305)	30 seconds	No data	V5	No data	No data	No data	T wave changes were seen in 73/305 (24%) of subjects
Lary & Goldschlager, 1974 (n = 46)	40-90 seconds	No data	12 lead	No data	No data	No data	T wave changes were seen in 7/46 (15%) of subjects
Thompson, 1943 (n = 25)	90 seconds	No data	I, II, III	No data	No data	No data	
Wasserberger *et al.*, 1956 (n = 350)	10-15 seconds	No data	12 lead	No data	No data	No data	T wave changes were seen in 37/350 (11%) of subjects

Table 1.2. Values represent mean changes from resting ECG in mV. * = <50% change, ** = >50% change, * = >100% change from the resting ECG. Studies are ranked in order of those that demonstrate the largest T wave ST segment changes (largest first).**

It is possible that ECG changes were caused by inconsistent inflation volumes that occur during voluntary hyperventilation (Rutherford *et al.*, 2005). This is indicated in studies where changes in T wave amplitude were matched by proportional changes in the R wave (table 1.2) (Barker *et al.*, 1939; Scherf & Schlachman, 1947; Christensen, 1946).

Patients with coronary artery disease

Studies examining the effects of hypocapnia induced by voluntary hyperventilation on the ECG of patients with coronary artery disease have inconclusive findings. This is likely to be caused by the problems associated with voluntary hyperventilation (stated in section 1.2.2). Clinically significant ST segment and T wave changes associated with ischemia have been reported during voluntary hyperventilation (for 30 seconds – 5 minutes) in patients with coronary artery disease (Kemp & Ellestad, 1968; Lary & Goldschlager, 1974; Joy & Trump, 1981; Ardissino *et al.*, 1987). In these studies, clinically significant ECG changes are referred to as ST segment elevation/depression of >0.1mV and T wave inversion (<-0.1mV). Hyperventilation is described but the degree of hypocapnia was not measured or quantified. These findings were not consistent in all patients tested. In 114 patients, Kemp & Ellestad (1968) reported 11 cases (10%) where voluntary hyperventilation caused ischemic T wave changes. Others have demonstrated clinically significant ST segment and T wave changes in up to 70% of patients suffering from known coronary artery disease (diagnosed by coronary angiography) during voluntary hyperventilation (Jacobs *et al.*, 1974; Joy & Trump, 1981; Ardissino *et al.*, 1987).

1.2.4. Mechanical hyperventilation and the ECG

The effects of hypocapnia, induced by mechanical hyperventilation, on the ECG have only been successfully investigated in one previous study. Rutherford *et al.*, (2005) demonstrated a statistically significant decrease in T wave amplitude (of 0.1mV) in 13/15 healthy subjects (87%) during hypocapnia (20 mmHg), induced by mechanical hyperventilation. They used a digital ECG recording system that averages two minutes of recorded ECG waveforms and demonstrated a reduction in T wave amplitude in lead I, independent of R wave changes. It

was suggested that this T wave reduction was caused by a decrease in O_2 availability in the myocardium as a result of hypocapnia induced coronary vasoconstriction and decreased O_2 dissociation into the tissues. By inducing hypocapnia with mechanical hyperventilation, tidal volume and breathing frequency could be accurately controlled and the effects of varied chest inflation on the ECG were eliminated. These effects were demonstrated in a single lead ECG with the limb electrodes placed in the modified electrode placement. It is unclear whether this modified electrode placement affected the wave amplitudes recorded in this study.

1.3. Summary & thesis objectives

The main objective of this thesis investigation was to study the effects of hypocapnia on cardiac electrical activity and heart function in normal subjects. In addition, it was of interest to study the effects of hypocapnia in patients suffering from coronary artery disease with stable angina to establish whether this technique has any value as a diagnostic test. The effects of hypocapnia on both normal subjects and patients were investigated using mechanical ventilation as described by Rutherford *et al.,* (2005). Since Rutherford *et al.,* (2005) used a modified electrode placement, it was of interest to establish to what degree this affects the ECG waveform compared to the standard electrode placement. Much debate still exists as to the extent of ECG changes that occur. Therefore, the first aim of this thesis investigation was to identify to what degree the modified electrode placement causes changes in the ECG waveform.

The 12 lead ECG allows the regional effects of hypocapnia to be assessed across the left ventricle. The effects of hypocapnia, induced by mechanical hyperventilation on the 12 lead ECG have never been fully assessed. The second objective of this thesis investigation was to

repeat the method of inducing hypocapnia by mechanical hyperventilation (used by Rutherford *et al.*, 2005) and measure its effects on cardiac electrical activity using a 12 lead ECG in normal subjects. It was hoped that this would confirm the findings of Rutherford *et al.*, (2005) and provide new information on the overall effects of hypocapnia across the heart. In addition to measuring R and T wave amplitudes, additional measurements of ST segment height and QTc interval duration were made and used to indicate whether ECG changes during hypocapnia were of ischemic origin. Echocardiograms were also recorded as a additional measure of heart function, to confirm any ischemic ECG changes seen with electrocardiography.

In the event that small, potentially ischemic ECG changes did occur during hypocapnia, normal subjects were also hyperventilated in 15% inspired O_2. Inspired hypoxia has been used previously to induce myocardial ischemia and ECG changes in both normal subjects and patients suffering from coronary artery disease (Katz *et al.*, 1934; Levy *et al.*, 1938; 1939; 1941; Barach *et al.*, 1941; Turner & Morton, 1952; Haarstad & Broch, 1958; Broch, 1972b). These effects were only evident when inspired O_2 was at 7.5-10% and O_2 saturation fell below 70% (Haarstad & Broch, 1958). Hypoxia of this severity is uncomfortable and has a number of adverse side effects (Stewart & Carr, 1954). In the present investigation, it was of interest to see whether a safe, comfortable level of inspired O_2 (equivalent to the O_2 inspired from air at an altitude of 6,700 feet [Ward *et al.*, 2000] or on routine commercial aircraft flights [Cottrell, 1988]) would be enough to augment any potentially ischemic ECG changes seen during hypocapnia in 21% inspired O_2.

The final study of this thesis investigation was a pilot study looking at the affects of

hypocapnia, induced by mechanical hyperventilation, on the ECG of patients with known coronary artery disease. The aim of this final investigation to was see if consistent, clinically significant ECG changes, similar to those seen during other provocative tests for coronary artery disease, can be induced by hypocapnia.

The following chapter will consider the general methods of experiments conducted in this thesis investigation. Subsequent self contained chapters will examine each aim of the thesis, and methods of these studies will only be summarised. The final chapter of the thesis will summarise the main findings of the thesis investigation, the clinical implications, the main strengths and limitations of the studies conducted and what these findings mean for future experiments conducted in this area.

Chapter 2

General Methods

2.1. Proposed experiments

1) Since Rutherford *et al.*, (2005) used a modified electrode placement to compare the effects of hypocapnia on the ECG, the effects of modifying the ECG electrode placement on the ECG waveform was investigated. Wave amplitudes recorded in both standard and modified electrode placements were compared and quantified in the context of the most up to date clinical guidelines on normal ECG waveforms.

2) The effects of hypocapnia on the single lead ECG in normal healthy subjects were confirmed using the methods of Rutherford *et al.*, (2005). In addition, the effects of hypocapnia on overall cardiac electrical activity were examined using a 12 lead ECG and global heart function using echocardiography.

3) Subjects were hyperventilated in 15% inspired O_2 to see whether this accentuated ECG changes during hypocapnia in 21% inspired O_2.

4) The effects of hypocapnia, induced by mechanical hyperventilation, on the ECG of patients suffering from coronary artery disease were investigated in a final pilot study.

2.2. Participants

The number of subjects required for each experiment was estimated on the basis of the findings of Rutherford *et al.*, (2005). They found statistically significant ECG changes of 0.1mV in 15 normal healthy subjects during mechanical hyperventilation in hypocapnia. Similar changes were anticipated in this thesis investigation and therefore an attempt was made to recruit at least 15 subjects for each experiment. For experiments comparing standard

and modified electrode placements and those studying the effects of hypocapnia on the ECG and echocardiogram, it was possible to study 18 normal healthy subjects, aged 24 ± 3 years old (20-30 years old) (13 male). In experiments studying the effects of hypocapnic hypoxia on the ECG, larger wave amplitude changes were anticipated and therefore fewer subjects were required. Experiments were completed in nine normal healthy subjects, aged 23 ± 2 years old (20-26 years old) (8 male). All experiments in mechanical hyperventilation conducted in the same subjects were carried out on separate days. All normal healthy subjects were undergraduate or postgraduate students from the School of Sport & Exercise Sciences at the University of Birmingham. All subjects were free from any known cardiovascular and respiratory disease and gave informed consent to participate. All experiments were approved by the Walsall Local Research Ethics Committee.

For experiments studying the effects of hypocapnia on the ECG of those suffering from coronary artery disease, eligible patients were identified by a consultant cardiologist at the University Hospital Birmingham. Patients were considered eligible for participation if they had undergone ECG exercise stress testing and had a normal ECG at rest with ischemic changes on exertion. Identified patients (and normal subjects) were excluded if they suffered from asthma, epilepsy, diabetes, renal failure, morbid obesity or had suffered from a cold, flu, sore throat, blocked nose, nose bleed or earache within the last seven days. Once identified, patients were contacted by phone and invited to attend an informal, introductory visit to the hospital laboratory where the experimental procedure was explained and informed consent was received. For every patient identified, permission for their participation was obtained from their GP. Over a period of two years, 16 patients were identified as eligible to participate in the experiment. Four of these patients (aged 61 ± 8 years old [42-74 years old, 4 male])

consented to take part and completed the full experimental protocol. In five patients, permission to participate was denied by their GP. The remaining seven patients declined to participate for personal reasons.

All four patients recruited had undergone a coronary angiogram to determine the extent of their disease and were awaiting either coronary angioplasty or a coronary artery bypass graft after participation in the experiment was complete. All patients were given a medical examination prior to participation in the study by the consultant cardiologist. Full patient details and past medical history can be seen in the appendix.

In each experiment, subjects lay semi-recumbent on a bed and were encouraged to listen to music or the radio through headphones to enable them to relax. In experiments using mechanical hyperventilation, subjects were given sufficient opportunity to acclimatise to the technique during preliminary visits and data were not collected until they had confirmed that they felt comfortable with the procedure. In addition to the 18 normal subjects who did participate in experiments involving mechanical hyperventilation, two who were unable to relax during mechanical hyperventilation were withdrawn from the study.

2.3. Mechanical ventilation

For experiments using positive pressure mechanical hyperventilation to induce hypocapnia, subjects were connected to a mechanical ventilator (Engstrom Erica II) via a rubber face mask which was attached to the face with head-straps. For experiments using hypocapnic hypoxia, this ventilator was replaced by an updated model (Däger Evita II), with the same features as

the previous model. Each subject was ventilated with disposable airway filters and sterilised masks. The ventilators were programmed with the same settings for all experiments.

The mechanical ventilator was set to deliver hyperventilation by the same method as Cooper *et al.,* (2004). Subjects were ventilated in synchronised intermittent mandatory ventilation mode (SIMV) via intermittent positive pressure ventilation (IPPV) which ventilates at a preset frequency and tidal volume but allows subjects to spontaneously trigger inspiration if necessary (Shneerson, 1996). If no breath is initiated by the subject, a mandatory breath is delivered at a predefined breathing frequency and tidal volume. During spontaneous breathing, contraction of the diaphragm and respiratory muscles increases the size of the thoracic cavity causing a negative intrathoracic pressure which is lower than atmospheric pressure. Air flows along the resulting pressure gradient and into the lungs (inspiration). During IPPV, a positive pressure is created in the ventilator facemask which is greater than the intrathoracic pressure. This air pressure is sufficient to overcome the elastic and flow resistive properties of the lungs and chest and create a pressure gradient from the upper airways to alveoli (Hillman, 1986). As in spontaneous breathing, expiration during IPPV is a passive process caused by the elastic recoil of the lungs.

The preset values for breathing frequency and tidal volume used in these experiments were higher than average breathing rates at rest. Cooper *et al.,* (2004) found that a constant breathing frequency of 16 br.pm and a tidal volume of ~1.3 litres achieves a reduction in $PetCO_2$ to approximately 20mmHg. During preliminary experiments, in which ECG data were not recorded, tidal volume was adjusted in each subject to identify the level necessary to

induce a drop in $PetCO_2$ of 20mmHg. When ECG data were being recorded, breathing frequencies and tidal volumes were kept constant at all stages of the experiment.

Subjects were hyperventilated in hypocapnic (low $PetCO_2$, ~20mmHg), normocapnic (normal $PetCO_2$, ~40mmHg) and hypocapnic hypoxic conditions (low inspired O_2, 15%, and low $PetCO_2$, ~20mmHg). Hypocapnia was achieved by ventilating subjects in room air. Because breathing frequency and tidal volume were set above normal resting values (frequency at rest = 8-12 br.pm; tidal volume at rest = ~0.8 litres), an imbalance in the proportion of CO_2 produced and the proportion of CO_2 exhaled occurred. This caused $PetCO_2$ to fall. If $PetCO_2$ decreased below 20mmHg, air from a cylinder containing 5% CO_2 in medical air (21% O_2, 79% N_2, *i.e.* room air) was added to inspired air to return $PetCO_2$ to 20mmHg. $PetCO_2$ was not allowed to fall below 20mmHg because at levels below this level, paraesthesiae and tetany have been shown to occur (Macefield & Burke, 1991). A $PetCO_2$ of 20mmHg typically took 10-15 minutes to induce. Once $PetCO_2$ was stable at the desired level, subjects were ventilated for a further 10 minutes before ECG or echocardiographic recordings were made.

Because the preset breathing frequencies and tidal volumes were constant throughout the experiment, $PetCO_2$ always fell to 20mmHg when subjects where ventilated in normal room air. To enable assessment of the ECG in baseline conditions (normocapnia), CO_2 was added to inspired air from a cylinder containing 5% CO_2 in 95% medical (room) air. To maintain normal $PetCO_2$, the percentage of CO_2 in inspired air was adjusted by a dial on the ventilator head stage. This elicited complete control of $PetCO_2$ throughout each experiment. Because resting $PetCO_2$ varies between individuals, baseline normocapnic conditions were adjusted for each subject based on measurements of $PetCO_2$ made during spontaneous breathing prior to

mechanical hyperventilation. Therefore, during normocapnia, $PetCO_2$ was not fixed to 40mmHg. Inspired O_2 was maintained at 21% (room air) throughout all experiments except in those which required reduced O_2 in inspired air. Once $PetCO_2$ was stable at the desired baseline level, subjects were ventilated for a further 10 minutes before ECG or echocardiographic recordings were made.

In hypocapnic hypoxia conditions, the 5% CO_2 in medical (room) air cylinder was replaced by a cylinder containing 15% O_2 in 85% N_2. Subjects breathed this hypoxic gas mixture at the same breathing frequency and tidal volume as before, without the addition of CO_2. This caused $PetCO_2$ to decrease to ~20mmHg. After $PetCO_2$ had been reduced sufficiently, subjects were ventilated in hypocapnic hypoxia for 10 minutes before ECG recordings were made.

2.4. Measuring instantaneous $PetCO_2$

$PetCO_2$ was recorded from expiration by infra-red spectrography using an in-line capnograph (Hewlett Packard 78354A). This capnograph shines infra-red light through an optical window, containing a sample of expired air. CO_2 absorbs infra-red light with a wavelength of 4.3μm. The amount of infra-red light absorbed at this wavelength is measured by an infra-red detector and this is compared with a known standard value for absorption of resting concentrations of CO_2.

In the present studies, $PetCO_2$ was used as a non-invasive indicator of the partial pressure of CO_2 in arterial blood ($PaCO_2$). $PetCO_2$ was taken as the plateau at the peak of the expiratory waveform from each breath. The advantage of using $PetCO_2$ to indicate $PaCO_2$ is that it

avoids need to take invasive, arterial blood samples and provides an instantaneous measure which can be used to control $PaCO_2$ with great sensitivity.

At rest in healthy individuals, $PetCO_2$ indicates $PaCO_2$ accurately to within 2-5mmHg (Bhavani-Shankar *et al.*, 1992; Frakes, 2001). Intra-patient values and changes in $PetCO_2$ are therefore considered reliable indicators of $PaCO_2$ (Frakes, 2001). This is satisfactory because within-subject changes in $PaCO_2$ were of primary interest in the present study. Capnography was used in resting normal subjects and stable patients who did not suffer from any respiratory or neurological disorders. Therefore, $PetCO_2$ was considered an acceptable measure of intra-subject changes in $PaCO_2$.

The Hewlett Packard 78354A capnograph was routinely calibrated by placing reference cells containing 0 mmHg and 56.2 mmHg of CO_2 into the optical sampling window and measuring the recorded values.

2.5. Measuring instantaneous blood pressure

Blood pressure was measured continuously throughout each experiment by a finger photoplethysmograph (Finapres 2300, Ohmeda, Englewood, CO, USA). The Finapres measures continuous blood pressure by measuring arterial diameter in the finger with an infrared photoplethysmograph. This information is relayed to an electropneumatic servosystem which inflates a finger cuff (placed on the middle finger) to a set point pressure which is halfway between intra arterial systolic and diastolic pressures (Imholz *et al.*, 1998). Changes in vessel diameter caused by changes in intra arterial pressure are detected by the infrared photoplethysmograph and the cuff pressure is adjusted accordingly. The transmural

pressure (pressure difference across the arterial wall in the finger) therefore remains constant (at zero) and thus the cuff pressure is the same as the intra arterial pressure giving a continuous measure of systolic, diastolic and mean arterial blood pressure (Imholz *et al.*, 1988).

The Finapres automatically calibrates itself every ~70 beats (for 2-3 beats) (Imholz *et al.*, 1990) to compensate for changes in finger size, packed cell volume, colour of the blood, smooth muscle tone and red blood cells washed out of the microcirculation under the cuff (Imholz *et al.*, 1988).

The Finapres produces an acceptable, continuous estimate of relative changes in intra arterial pressure in normal resting subjects and patients (Imholz *et al.*, 1988; van Egmond *et al.*, 1985). However, when Finapres measurements are compared to intra-arterial pressure measured in the brachial artery, the Finapres may overestimate (by 7 ± 11mmHg) or underestimate absolute values (by 13 ± 10mmHg) (Wesseling *et al.*, 1985; Imholz *et al.*, 1988; van Egmond *et al.*, 1985; 1990). In the present experiments, all subjects were known to be normotensive and only relative changes in blood pressure were of interest. To maximise the accuracy of the Finapres, an attempt was made to meet the following conditions during its use;

- Subjects should be resting
- Ambient temperature should be >22°C
- The correct cuff size should be identified and used
- The hand should be at the same level as the heart

– Hands should be kept warm to ensure adequate blood supply to the fingers

(Imholz *et al.*, 1998)

In the present study, all subjects and patients were laid semi-recumbent on a bed with their hand elevated (as appropriate) to the same level as the heart. In some cases, subjects had very cold hands. In these instances an attempt was made to warm the hand by placing it on a bean bag (heated in a microwave) and wrapping in a blanket.

2.6. Measuring air flow in spontaneous breathing

For experiments using 15% inspired O_2, the effects of inspiring 15% O_2 on breathing frequency, tidal volume and metabolic rate were assessed. Spontaneous breathing characteristics were recorded using an ultrasonic phase shift flowmeter (BDRL Flowmetrics, Birmingham, UK). The flowmeter has two ultrasonic transducers which measure the phase shift in the ultrasonic signal that occurs during each respiratory cycle. This is converted into a voltage for the mean velocity of air through the flowmeter tube. Before its use in each experiment, the flowmeter tube was placed vertically against a flat surface so that no air could flow through it. The flowmeter readings were checked and if necessary, manually adjusted to zero.

Subjects wore a nose clip and breathed through a three-way mouth piece connected to the flowmeter and an in-line capnograph (Hewlett Packard 78354A) (for measuring end-tidal CO_2) (figure 2.1). A Douglas bag (containing a certified gas mixture of 15% O_2 in 85% N_2) with a three-way valve was connected to the flowmeter and this allowed the experimenter to

switch the subject's inspired air between the Douglas bag and room air without their knowledge.

Figure 2.1. Flowmeter apparatus

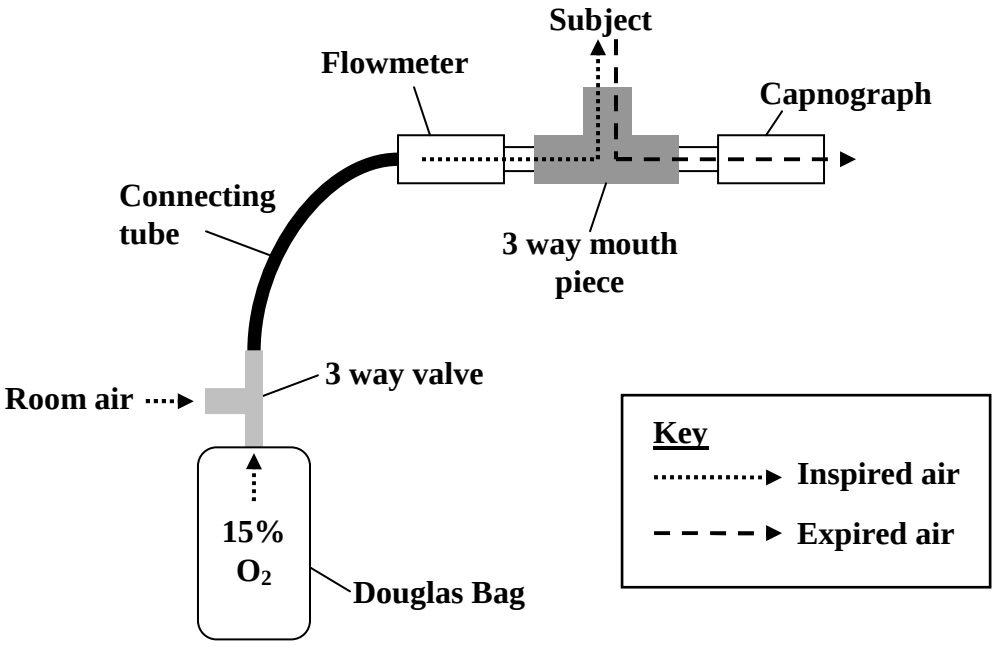


Figure 2.1. Apparatus setup for experiments using flowmeters and Douglas bags

Data were recorded by the Spike2 data acquisition software via a CED power 1401 PC interface (Cambridge Electronic Design, UK). Data from the flowmeter was used to calculate breathing frequency and tidal volume. The flowmeter was calibrated using a volume syringe which passes known volumes of air through the apparatus. The flowmeter was calibrated with 0.2 litre, 0.5 litre, 0.8 litre and 1.0 litre volumes of air. This was repeated 5 times. A mean of all the measured values for each known volume was calculated and compared to the expected values. Any discrepancies between actual and measured values were corrected by a calibration factor that was calculated by dividing the actual value by the mean recorded value. This calibration factor was subsequently used to correct data recorded from human subjects.

2.6.1. Airflow Analysis

Data from the flowmeter and capnograph were recorded on the Spike2 data acquisition software and analysed offline. The flowmeter produces a continuous waveform representing the flow of air during inspiration and expiration. Inspiration is represented by a flow peak on the breathing trace. Spike2 allows the partial pressure of CO_2 in expired air to be recorded simultaneously and the resulting peak partial pressures on the CO_2 trace correspond to each expiratory phase on the breathing trace. A Spike2 data analysis script was used to calculate instantaneous breathing frequency, tidal volume, minute ventilation (V_E), instantaneous CO_2 production (VCO_2) and V_E/VCO_2 ratio.

Instantaneous breathing frequency

The Spike2 analysis script was programmed to measure the interval duration of each respiratory cycle from the beginning of inspiration to the end of expiration for each breath throughout the recording period. Instantaneous breathing frequency was calculated by dividing 60 (seconds) by the duration of each respiratory cycle. Calculated values were manually checked by measuring duration of respiratory cycles from different data files and dividing 60 by this measured value.

Tidal volume

The total area under each inspiratory peak (on the flow trace) was calculated as a measure of tidal volume. The flowmeter was zeroed before each experiment by blocking one end of the sampling tube (causing zero flow through the tube) and adjusting the recorded value to zero on the flowmeter head stage. Data was not standardised and was presented as volumes of gas at body temperature and atmospheric pressure, saturated with water (BTPS). Volume

measurements were calibrated using a volume syringe which passes known volumes of air through the apparatus. Measured volumes were compared to these known volumes.

Instantaneous minute ventilation

Instantaneous V_E was calculated by multiplying the tidal volume of each breath by its instantaneous breathing frequency.

Instantaneous CO_2 production

The Spike2 script calculated the instantaneous CO_2 production in each expired breath from the tidal volume recorded on the flow trace and the partial pressure of CO_2 in expired air recorded by the capnograph. This script converted the partial pressure of CO_2 in expired air into a % of air (equation 2.1) and multiplied this by the tidal volume of each breath. Data was standardised at a temperature of 0°, barometric pressure of 760 mmHg and unsaturated with water (dry) (STPD) using equation 2.2. Instantaneous VCO_2 was used as an indicator of metabolic rate.

Equation 2.1.

$$\% \text{ of gas in air} = \left(\frac{\text{Partial pressure of gas in air}}{\text{Barometric pressure}} \right) \text{X } 100$$

Equation 2.2.

$$\text{Volume at STPD} = \left(\frac{(\text{Volume}_{[BTPS]} \text{ X Barometric pressure}_{[BTPS]}) - pH_2O_{[BTPS]}}{760} \right) \text{X } \left(\frac{273}{(273 + \text{ambient temperature})} \right)$$

V_E/VCO_2 ratio

In was of interest to know whether changes in V_E were due to changes in metabolic rate or changes in inspired O_2. Changes in V_E caused by alterations in inspired O_2 are evident by the presence of hypo/hyperventilation. This can be estimated using the V_E/VCO_2 ratio which is calculated by dividing V_E by VCO_2. An increase in the V_E/VCO_2 ratio indicates that the subject is hyperventilating. A decrease in the V_E/VCO_2 ratio indicates that the subject is hypoventilating.

2.7. Measuring O_2 saturation

For experiments where 15% O_2 was inspired, a pulse oximeter (N-200 series oximeter, Nellcor) was used to measure O_2 saturation in arterial blood. Pulse oximetry was used because it allows accurate estimations of arterial O_2 saturation, non-invasively (Yelderman & New, 1983). In addition, it provides an instantaneous measure of O_2 saturation which allows changes over time to be investigated.

The pulse oximeter uses a finger cuff containing two light emitting diodes (LEDs) which transmit red light at a wavelength of 0.66μm and infrared light with a wavelength of 0.94μm (Yelderman & New, 1983) through the arteries in the finger. Light of these wavelengths is absorbed by oxyhaemoglobin and deoxyhaemoglobin. The amount of light absorbed at each wavelength is calculated by the Beer-Lambert law which relates the amount of transmitted light from the LED to the amount of light absorbed and not absorbed by haemoglobin (Grace, 1994). An ‘R’ ratio is calculated from absorbance values of each wavelength using equation 2.3. O_2 saturation is calculated from the R ratio by relating the ratio to a known equivalent O_2 saturation.

Equation 2.3.

$$\text{R ratio} = \frac{AC_{0.66\mu m} / DC_{0.66\mu m}}{AC_{0.94\mu m} / DC_{0.94\mu m}}$$

A = absorbance of light a predetermined wavelength, C = concentration of haemoglobin in arterial blood, D = distance of transmitted light from the LED

Measurements made within the range of 100-70% saturation correlate well with measures of O_2 from arterial blood samples (Yelderman & New, 1983). In standard pulse oximeters, it is thought that any measured values below 80% should be taken with caution (Grace, 1994). In the present study, saturation did not fall below 90% and thus values were considered acceptable.

The pulse oximeter is calibrated by the manufacturer using healthy control subjects and is said to be accurate to within ± 2% (Kidd & Vickers, 1989). In clinical or research settings, pulse oximeters cannot be manually adjusted by an experimenter (Grace, 1994; Huch *et al.*, 1988). In the present experiments, only healthy subjects were used and therefore, the manufacturer's calibration was considered acceptable.

2.8. Measuring plasma electrolytes

During experiments in hypocapnia venous blood samples were taken from 14 normal subjects and analysed for plasma electrolyte concentrations and blood pH. These 10ml samples were taken by a trained nurse from the antecubital vein, at the end of normocapnia and hypocapnia during mechanical hyperventilation. Each sample was analysed for blood pH and ionized K^+ and Ca^{2+} concentrations using a Rapid Lab 865 blood gas analyzer. The Rapid Lab 865 analyzer automatically calibrated itself at regular intervals specified by hospital staff for

clinical use. Ionized electrolyte concentrations were measured because it is ionized component of electrolyte concentrations which have the greatest physiological effects around the body (Calvi & Bushinsky, 2008).

2.9. Electrocardiography

2.9.1. Background in electrocardiography

The ECG monitors the electrical activity of the heart, non-invasively. A standard ECG consists of 12 leads, each of which measures the electrical activity within the myocardium from 12 different electrical axes. The 12 lead ECG was used in the present investigation to study the effects of hypocapnia on cardiac electrical activity in normal subjects and patients suffering from coronary artery disease.

Myocardial excitation

Contraction of the heart is achieved by an electrical conduction system that propagates through the myocardium. The purpose of this conduction system is to co-ordinate contraction of the atria, followed by a sufficient delay before coordinated contraction of the ventricles. This delay maximises the flow of blood into the ventricles and subsequently around the body (Noble, 1975). Stimulation of this conduction system begins at the pacemaker sinoatrial node (SA node) (Noble, 1975).

The action potential generated in the SA node excites surrounding myocytes (cardiac muscle cells) in the atria causing them to contract almost simultaneously. This action potential travels through the atria until it reaches the atrioventricular (AV) node. Here, the signal travels down the bundle of His (including the left & right branch bundles) to the apex of the heart.

Ventricular systole is caused by contraction of the cardiac myocytes as the action potential travels through the ventricle walls via the Purkinje fibres (Noble, 1975).

In humans, the resting membrane potential in the SA node is -60mV. At the start of an action potential, gradual inflow of Na^+ along the Na^+ concentration gradient causes this membrane potential to rise (depolarisation) (Noble, 1975). When the membrane potential reaches a threshold value of -40mV, voltage regulated calcium channels open and Ca^{2+} flows down the Ca^{2+} concentration gradient into the cytosol. This causes the membrane potential to rapidly increase until there is no longer a concentration gradient between intracellular and extracellular Na^+ and Ca^+. At this point, K^+ channels open and K^+ flows out of the cell along its concentration gradient. This process is called repolarisation and restores the membrane to its resting potential of -60mV.

The action potential that occurs in cardiac myocytes differs from the pacemaker potential in the SA node. The resting membrane potential in a cardiac myocyte is -90mV and only changes when voltage-sensitive Na^+ channels are opened by an electrical stimulus from the SA node (Page, 1962). Na^+ enters the myocyte along its concentration gradient causing the membrane potential to increase to 30mV, at which point Na^+ channels close. Depolarisation is prolonged (plateaus) by the influx of Ca^{2+} through calcium channels. There, Ca^{2+} binds to troponin causing contraction of the myocyte via excitation-contraction coupling (interaction of actin and myosin filaments). At the end of the plateau, K^+ channels open and K^+ flows down its concentration gradient out of the cell restoring the resting membrane potential (Page, 1962). Excitation of one myocyte stimulates contraction of the adjacent myocyte (Wagner, 2008). This allows contraction of the entire muscle in a chain reaction directed by

the conduction pathway. The SA node reaches its threshold potential before that of the bundle of His, the Purkinje fibres and the cardiac myocytes. Therefore, it is the SA node which dictates the rate at which the heart beats (Noble, 1975).

The electrical activity occurring in each myocyte forms part of the total pattern of electrical activity across the myocardium which can be monitored using an ECG. The ECG is therefore a composite of electrical activity recorded in many different parts of the myocardium. This ECG waveform can be used to identify the different events of the cardiac cycle (figure 2.2).

Figure 2.2. Time points of electrical activity within the heart

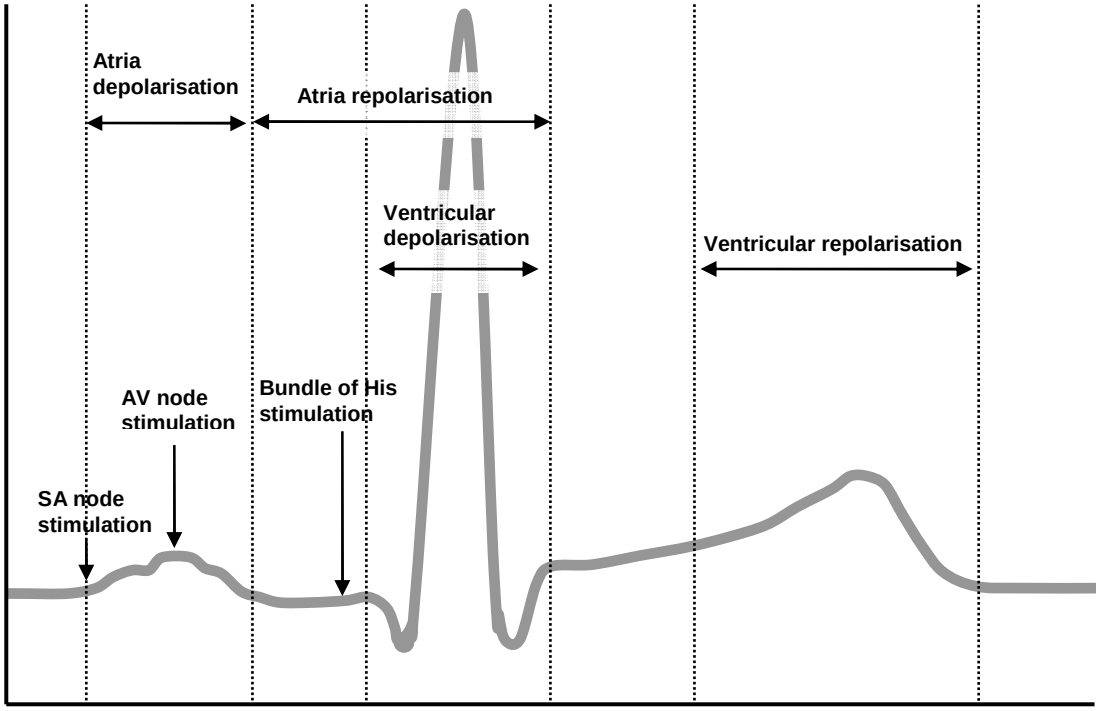


Figure 2.2 shows a typical ECG waveform (lead I) and the time points at which electrical events occur in the myocardium (adapted from Wagner (2008). *Marriott's Practical Electrocardiography*, 11th edition).

Figure 2.3. ECG wave amplitudes and durations

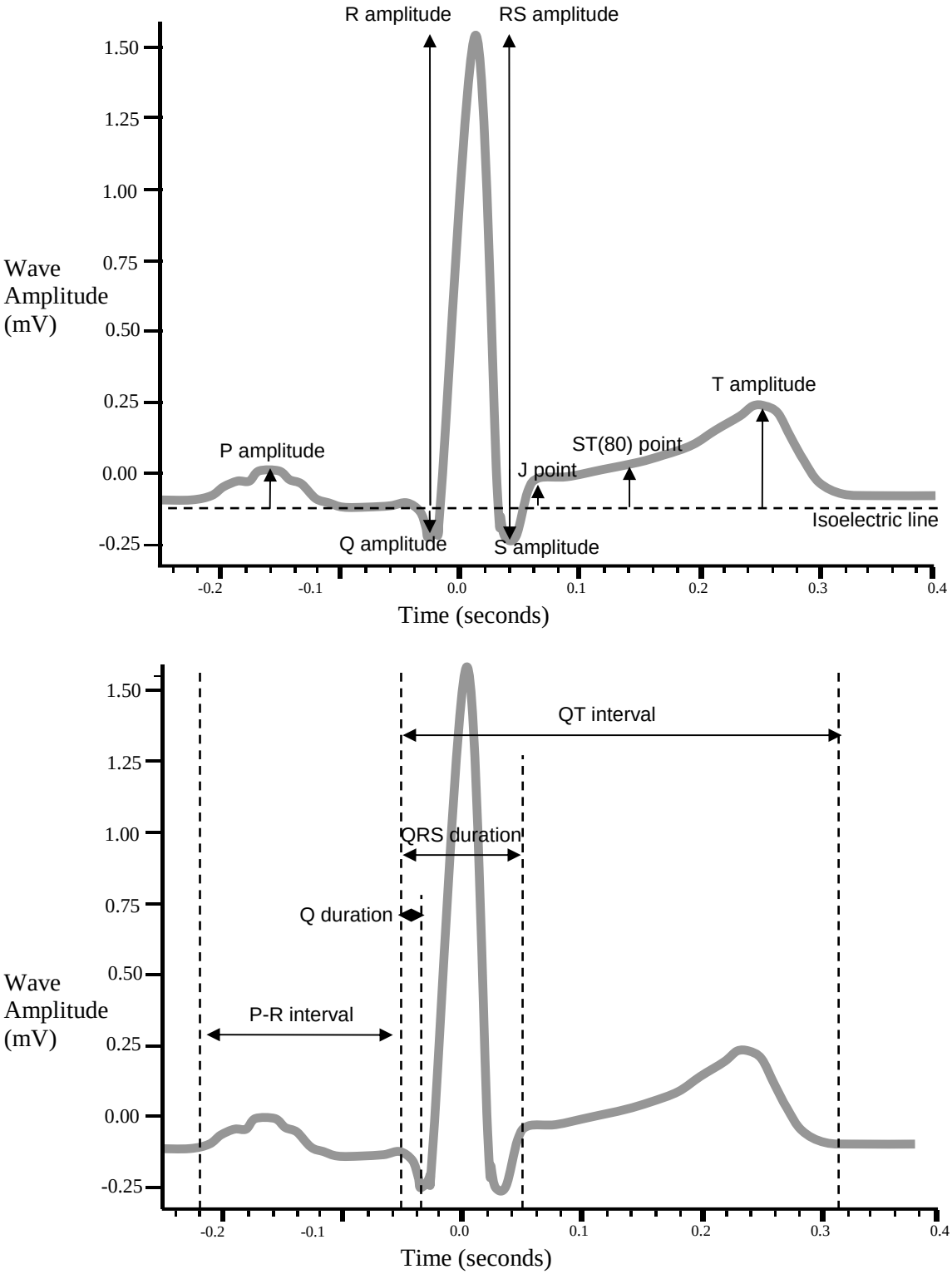


Figure 2.3. ECG wave amplitudes (measured from the isoelectric line) and interval durations. The level of the isoelectric line taken from the electrically neutral point from the end of the P wave to the beginning of the Q wave.

Firing of the SA node and atrial depolarisation is represented by the P wave (figure 2.2, 2.3) (Wagner, 2008). This is followed by atrial repolarisation, but this is hidden on the ECG trace by the events of the QRS complex, which occur further down the cardiac conduction pathway (Braunwald *et al.,* 2001). Stimulation of the AV node and the bundle of His occur during the P-R segment. This is followed by septal, apical and left & right ventricular depolarisation which are represented by the Q, R and S waves (figure 2.2, 2.3) (Wagner, 2008). The T wave represents the process of ventricular repolarisation (figure 2.2, 2.3). The QT interval therefore represents the duration of ventricular activation and recovery (figure 2.2, 2.3). Between the QRS complex and the T wave is the ST segment (figure 2.3). This represents an electrically neutral period between ventricular activation and recovery (figure 2.2) (Wagner, 2008).

Calculating the ECG

The ECG is based on the principles of Einthoven (1912), Wilson *et al.,* (1934) and Goldberger (1942). The standard 12 lead ECG is made up of 3 limb leads, 3 augmented limb leads and 6 chest leads derived from 10 electrodes placed in standardised positions (figure 2.4). Each lead views the same electrical events in the heart from a different position (figure 2.5). Leads I, II and III make up the three limb leads of 'Einthoven's Triangle' (Einthoven, 1912) (equation 2.4). These bipolar leads are derived from 4 electrodes placed on the limbs (figure 2.4) and represent the potential difference between two of these limb electrodes, creating three different electrical views of the heart across the frontal plane (figure 2.5). The electrical potential at each electrode is calculated from the difference between the exploring electrode and a reference electrode placed on the right leg (RL). Lead I is calculated from the potential difference between the left and right arm electrode. Electrical currents in the cardiac muscle moving towards the positive electrode (left arm [LA]) are depicted as a positive

Figure 2.4. Standard ECG electrode placement

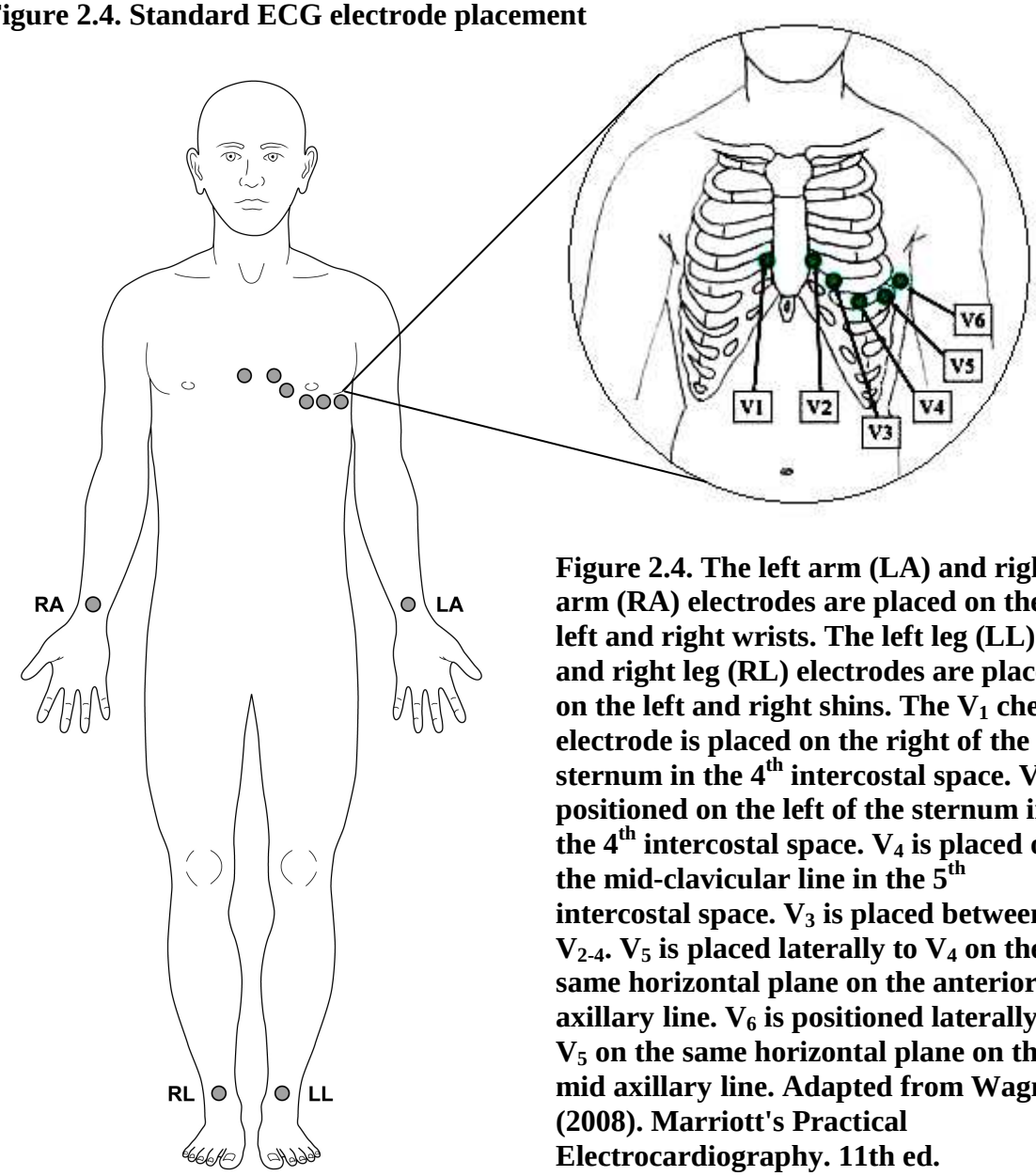


Figure 2.4. The left arm (LA) and right arm (RA) electrodes are placed on the left and right wrists. The left leg (LL) and right leg (RL) electrodes are placed on the left and right shins. The V_1 chest electrode is placed on the right of the sternum in the 4th intercostal space. V_2 is positioned on the left of the sternum in the 4th intercostal space. V_4 is placed on the mid-clavicular line in the 5th intercostal space. V_3 is placed between V_{2-4}. V_5 is placed laterally to V_4 on the same horizontal plane on the anterior axillary line. V_6 is positioned laterally to V_5 on the same horizontal plane on the mid axillary line. Adapted from Wagner (2008). Marriott's Practical Electrocardiography. 11th ed.

deflection on the ECG trace. Electrical currents moving towards the negative electrode (right arm [RA]) are illustrated as a negative deflection. This is the case for all of Einthoven's limb leads where Lead II is the potential difference between the left leg (LL) and the RA electrodes and Lead III is the difference between the LL and LA electrodes. Kirchhoff's law states that in a closed electrical circuit, such as the equilateral triangle formed of Einthoven's limbs

Figure 2.5. ECG electrical planes and axes

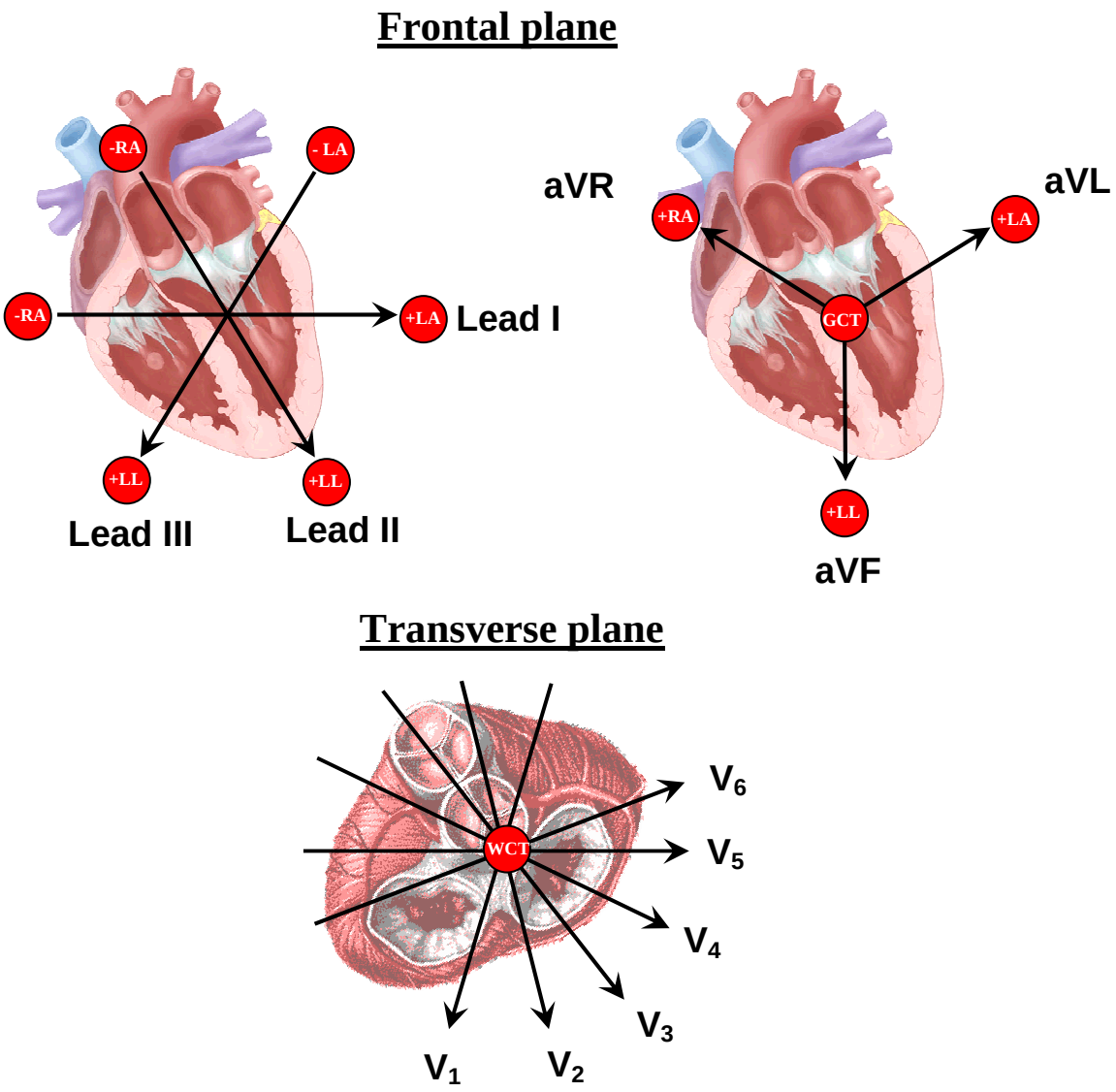


Figure 2.5. Electrical axes of the heart on the frontal and transverse planes. The arrows of each electrical axis point in the direction of the positive pole (electrode) of each lead. GCT = Goldberger central terminal (negative pole), WCT = Wilson central terminal (negative pole). Adapted from Wagner (2008). Marriott's Practical Electrocardiography. 11th ed.

leads, the sum of all the electrical current flowing in all directions must equal zero (Kligfield *et al.*, 2007). It is possible then, to calculate the wave amplitude at any point in the cardiac cycle in one limb lead from the same amplitudes in the other two limb leads. This theory defines Einthoven's Law which states, "the difference between the electrical tensions of

Leads I and II must be equal to the electrical tension of Lead III" (equation 2.5) (Einthoven, 1912).

Equation 2.4. - Einthoven's limb leads

$$\text{Lead I} = LA - RA$$

$$\text{Lead II} = LL - RA$$

$$\text{Lead III} = LL - LA$$

Equation 2.5. - Einthoven's law

$$\text{Lead III} = \text{Lead II} - \text{Lead I}$$

Leads aVR, aVL and aVF were derived from Wilson's original Vr, Vl & Vf leads (Wilson *et al.*, 1934). Leads Vr, Vl and Vf are unipolar leads that use the limb electrodes as exploring electrodes and a central terminal as a reference point. The central terminal is calculated as the mean of the electrical potential at each of the three exploring limb electrodes (equation 2.6). These leads have significantly smaller wave amplitudes compared to the limb leads because they include the exploring electrode within their own central terminal reference point. Including the electrical potential from the exploring electrode in the reference point causes it to be subtracted, in part, from the electrical potential of the exploring electrode when the lead is derived. This reduces the amplitude of the waveforms. Goldberger (1942) proposed a revised central terminal that does not include the extremity electrode from which the ECG lead is recorded. This produces an augmented electrical signal and is used to calculate the three 'augmented' limb leads (equation 2.7). These augmented limb leads monitor the electrical activity across the frontal plane of the heart and are calculated by creating an

indifferent electrode from two of the limb electrodes and using this as a negative reference to the remaining limb exploring electrode (equation 2.7).

Equation 2.6. - Wilson's central terminal

$$\text{Central terminal} = \frac{RA + LA + LL}{3}$$

Equation 2.7. - Goldberger's augmented limb leads

$$\text{Lead aVR} = \text{RA} - \left(\frac{LA + LL}{2}\right)$$

$$\text{Lead aVL} = \text{LA} - \left(\frac{RA + LL}{2}\right)$$

$$\text{Lead aVF} = \text{LL} - \left(\frac{LA + RA}{2}\right)$$

Equation 2.8. - The precordial leads

$$V_1 = V_1 - \left(\frac{RA + LA + LL}{3}\right) \qquad V_2 = V_2 - \left(\frac{RA + LA + LL}{3}\right)$$

$$V_3 = V_3 - \left(\frac{RA + LA + LL}{3}\right) \qquad V_4 = V_4 - \left(\frac{RA + LA + LL}{3}\right)$$

$$V_5 = V_5 - \left(\frac{RA + LA + LL}{3}\right) \qquad V_6 = V_6 - \left(\frac{RA + LA + LL}{3}\right)$$

The precordial leads are derived from the central terminal (equation 2.6) and 6 unipolar exploring electrodes (figure 2.4) that are placed across the chest to provide a view of the heart's electrical activity across the transverse plane (figure 2.5) (equation 2.8) (Wilson *et al.*, 1934). In this thesis, the limb and augmented limb leads are referred to as the frontal plane leads. The precordial leads are referred to as the transverse plane leads.

Gender Differences in the ECG

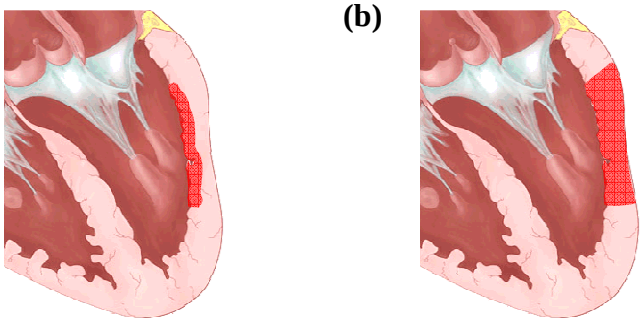
It is thought that ECG waveforms are altered in normal resting subjects of different ages and gender as a result of differing testosterone levels which affect the ventricular repolarisation phase of the cardiac cycle (Bidoggia *et al.*, 2000; Macfarlane, 2001; Surawicz & Parikh, 2002). In a study of 500 normal healthy subjects (250 male), male T wave amplitudes in the transverse plane leads were found to be to 0.3 ± 0.1mV ($P < 0.001$) larger than female T wave amplitudes (Bidoggia *et al.*, 2000). In addition, the height of the ST segment is also higher in men than women (0.3mV in men vs. 0.1mV in women in lead V_2, [no standard error or statistical analysis presented]) (Macfarlane, 2001). The gender specific effects on the ECG are most prominent in the transverse plane leads (V_{1-4}) (Bidoggia *et al.*, 2000) with the largest differences (of 0.2mV) in found in V_2 (Macfarlane, 2001). 'Male ECG characteristics' tend to appear in men aged >17 years and decline from >25 years (Surawicz & Parikh, 2002). The gender difference in T wave amplitude and ST segment height therefore decreases with age due to a decrease in wave amplitude in males (*e.g.* ST difference of 0.2mV in normals aged <29 years vs. 0.1mV in normals aged >50 years [Macfarlane, 2001]). These gender and age specific changes in T wave amplitude and ST height appear to coincide with changes in blood testosterone levels which, in men, rise during puberty and decline with old age (Surawicz & Parikh, 2002). Age specific changes are therefore less evident in women whose blood testosterone levels are much lower and more stable. In this thesis investigation, it was important to be aware of these gender and age differences in the ECG because they affect the clinical thresholds used to define the presence or absence of clinically significant changes in the ECG. Because both male and female subjects participated in all experiments, both non-specific and gender-specific clinical thresholds were used to analyse changes in the ECG.

Myocardial ischemia and the ECG

Myocardial ischemia causes changes in the propagation of electrical activity throughout the heart causing changes in the ECG waveform morphology. The clinical significance of these ischemic ECG changes depend on the location and severity of the ischemic episode (Wagner, 2008). If a myocardial cell becomes ischemic, it remains in a resting state in order to survive. This involves uncoupling from electrical activation by adjacent myocardial cells (Wagner, 2008). The lack of electrical activity in these areas results in an 'injury current' which shifts the electrical axis of cardiac activity away from the ischemic region (Wagner, 2008). This causes noticeable ECG changes such as T wave inversion (change in the polarity of the T wave) and ST segment depression below -0.1mV (Wagner *et al.*, 2009). Such changes represent mild ischemia in the subendocardial layer (innermost layer) of the myocardium (figure 2.6a). If the ischemia is transmural (affecting an entire region of myocardium caused by decreased O_2 supply resulting from total occlusion of the coronary artery [figure 2.6b]), the injury current is directed towards the affected region causing hyperacute T waves (> ~0.5 mV in the frontal plane leads and > ~1mV in the transverse plane leads) and ST segment elevation (>0.1-0.25mV) (2009; Wagner, 2008). Sustained transmural ischemia leads to myocardial injury and ultimately cell death (myocardial infarction).

Figure 2.6. Affected regions of the left ventricle during subendocardial and transmural ischemia in the lateral wall

(a) **(b)**



Subendocardial ischemia **Transmural ischemia**

T wave changes alone are not considered reliable indicators of ischemia because clinically significant T wave changes can occur in normal healthy subjects with normal coronary arteriograms (Taggart *et al.*, 1979) and sometimes fail to appear in patients suffering from myocardial ischemia (Wagner, 2008). However, it is thought that T wave inversion and hyperacute T waves, when followed by ST segment depression/elevation, represent the earliest phases of subendocardial/transmural ischemia (Birnbaum *et al.*, 1993; Wagner, 2001; Sclarovsky *et al.*, 1988; 2008). QT interval prolongation is also thought to provide an early indication of transmural myocardial ischemia (Kenigsberg *et al.*, 2007).

The 12 lead ECG enables the identification of the ischemic region within the heart and the occluded artery supplying that region. The left ventricle can be divided into septal, anterior, lateral and inferior walls (Wagner, 2008). Each ECG lead monitors the electrical activity in these ventricular walls. The distribution of coronary arteries across the left ventricle can vary from patient to patient. Typically, septal and anterior walls are supplied by the left anterior descending artery (LAD). The left circumflex artery (LCX) perfuses the lateral region and some portions of the anterior wall. The right coronary artery (RCA) and the posterior descending artery (PDA) supply the inferior wall of the left ventricle (Wagner, 2008). Therefore, an occluded coronary artery can be located by identifying the ECG leads in which ischemic changes occur and correlating that with the coronary arteries that supply that region of the heart. The nature of the ECG changes that occur depend on whether the myocardial ischemia is caused by an increase in myocardial O_2 demand or a reduction in O_2 supply. Diagnosis of ischemia is only indicated when ECG changes occur in 2 or more anatomically contiguous leads (Wagner *et al.*, 2009).

2.9.2. Recording the ECG

All ECGs were recorded using the apparatus described below. This apparatus allows the ECG to be recorded and measured without losing potentially important ECG information through unnecessary filtering. In addition, it allows continuous 12 lead ECG recordings which can be averaged using specially designed software. This software enables exact measurement of ECG wave amplitudes which are not influenced by human error or bias.

Two-minute ECG recordings were made after 10 minutes mechanical hyperventilation in each condition (*i.e.* normocapnia, hypocapnia, hypocapnic hypoxia). ECG waveforms were averaged across this two minute period (creating a single average waveform for ~120 cardiac cycles). This was done because it creates a waveform that is typical of the ECG trend but is not affected by individual anomalous beats. In all experiments, ECG leads were connected to Blue sensor electrodes (P-00-S/50, Ambu) which were used because they provide good adherence to the skin. To achieve the optimum recording of ECG signals with minimal impedance, the stratum corneum layer of the epidermis on the skin surface (layer of dead skin cells which do not conduct electricity well) was removed from all electrode sites using Nu-prep abrasion gel (Smith, 1984). Acetone was used to remove any skin oils that might increase contact impedance at the electrode site.

Analogue ECG data was recorded by two D360 patient isolated amplifiers connected to a power1401 CED system (figure 2.7). Each D360 patient isolated amplifier has an external unit with 8 analogue inputs. The electrical potential at each exploring electrode was recorded in reference to a common electrode placed on the right leg. The 3 limb electrodes were connected to the first D360 amplifier and the 6 precordial exploring electrodes were

Figure 2.7. ECG apparatus setup

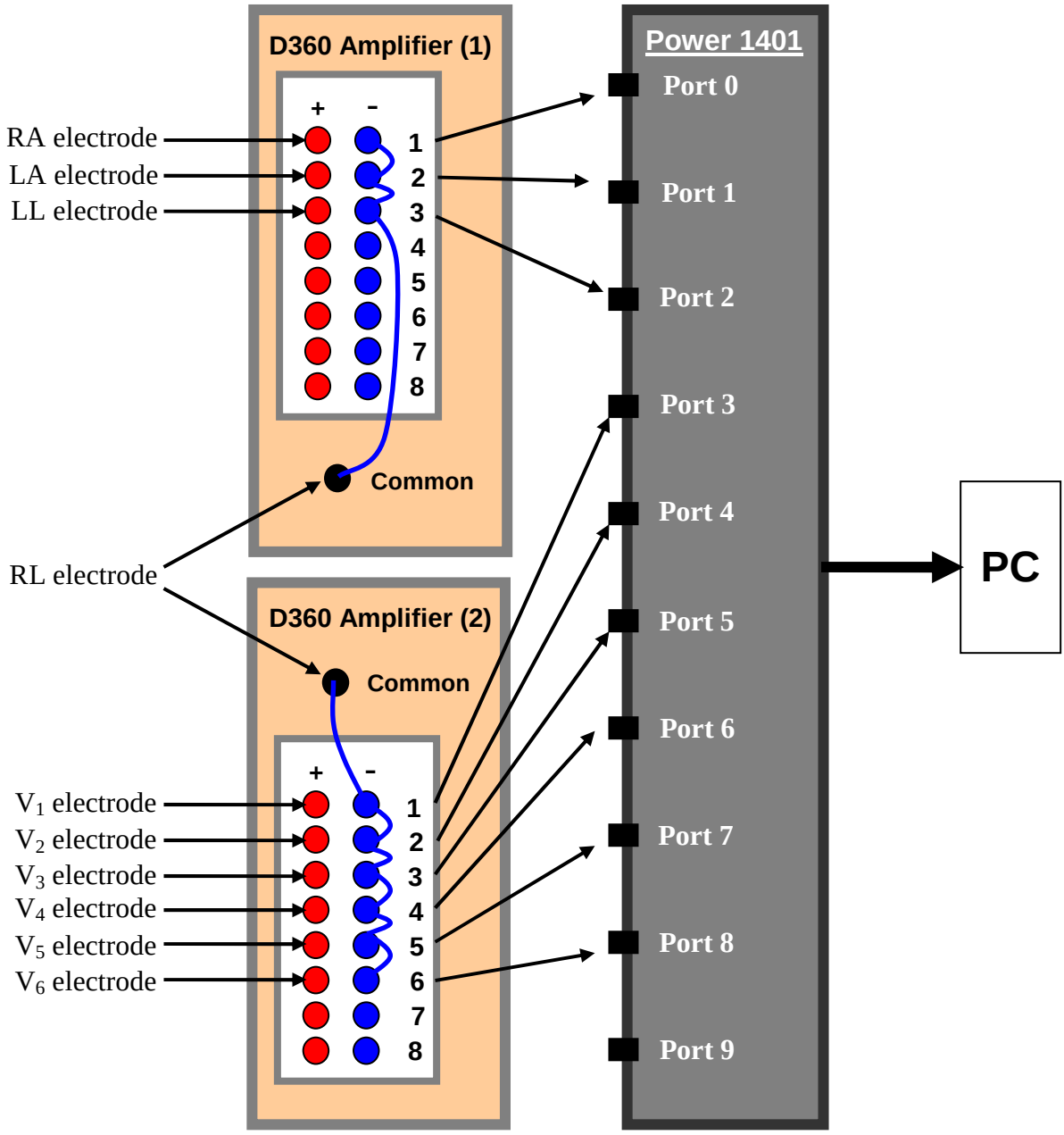


Figure 2.7. ECG electrodes are connected to a D360 amplifier and recorded as an analogue signal before being converted into a digital waveform by a power 1401 connected to a PC.

connected to the second (figure 2.7). The right leg electrode was connected to all 9 inputs. Each channel on the D360 was connected to an individual port on the power1401 CED digital converter. The power1401 digitises the analogue signals recorded from the ECG electrodes,

allowing them to be viewed on a PC using CED Spike2 data acquisition software. This apparatus enables several channels of data to be recorded simultaneously.

The ECG was recorded with a frequency bandwidth of 0.005-1000 Hz. This was set to ensure that all aspects of the true ECG signal could be recorded. Signals were sampled by the power1401 at a predetermined frequency which was set high enough to prevent distortion of the final digitally reconstructed signal. It is known that to prevent this aliasing, the minimum sampling rate of any signal should be at least twice that of the highest required frequency component (Nyquist, 2002). Thus, for a high frequency component of 1000 Hz, we sampled at 2500 Hz.

The ECG signal was recorded from each electrode without the application of high and low pass filters or 50 Hz notches. Standard digital ECG machines sample the ECG signal from electrodes on the body surface and apply high pass filters to the signal to eliminate baseline drift (caused by respiration) and low pass filters to eliminate muscle artefact and mains noise (Kligfield *et al.*, 2007). The low frequency cut-off is normally set to 0.5 Hz (corresponding to a heart rate of 30 bpm) but this can cause distortion to the ECG, particularly in the ST segment (Kligfield *et al.*, 2007). High frequency cut-offs of 150 Hz are considered acceptable in normal adults but these can eliminate higher frequency components of the QRS complex thus invalidating some wave amplitude measurements. Some clinical ECG machines contain a 50 Hz notch filter which targets and attenuates frequencies of 50 Hz to eliminate mains noise. However, the application of a 50 Hz notch to the data is a crude form filtering which can eliminate waveforms of adjacent frequencies which may contain important ECG information.

The recorded ECG signal contained 50 Hz mains noise and a harmonic series of this noise. This can distort the ECG signal making it difficult to identify the different waves of the ECG (figure 2.8). Offline power spectral analysis was performed on the recorded signal from each electrode to reveal the frequency components of this 50 Hz harmonic series. In figure 2.9a, these components are represented by the black bins and can be seen at frequencies of 50 Hz (×1), 100 Hz (×2), 150 Hz (×3), 250 Hz (×5), 350 Hz (×7) and 550 Hz (×11). This harmonic series was seen in signals recorded from each ECG electrode. Because the frequency of these harmonics was known, it was possible to remove them from the data using a Spike2 analysis script. This script creates a sine wave that matches the frequency of the harmonic series in the data every 5 sinusoidal cycles and subtracts it. This sine wave has the correct wave amplitude and phase shift and is calculated from a combination of sine and

Figure 2.8. Effects of 50 Hz mains noise on the on the ECG signal

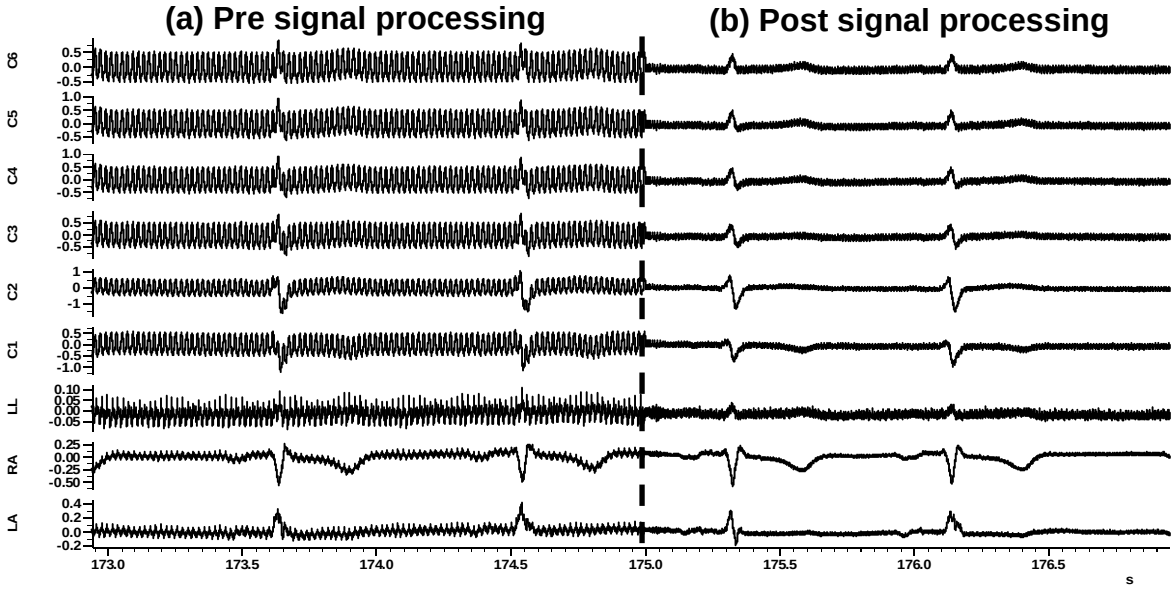


Figure 2.8. Raw data from one subject showing a) the effects the 50Hz harmonic series on the ECG signal recorded from each electrode and b) the ECG signal after the 50Hz harmonics have been removed.

Figure 2.9. Power spectral analysis of the ECG signal

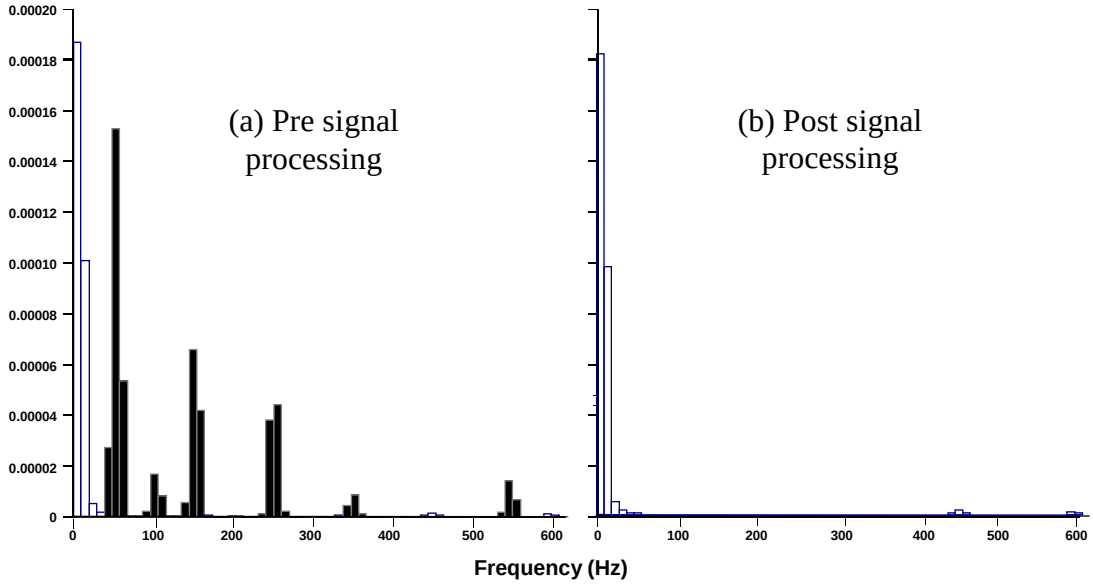


Figure 2.9. a) shows the different frequencies recorded in the ECG signal from the left leg electrode in one subject. The ECG frequencies are represented by the white bins (below 50Hz). The black bins represent the 50Hz mains noise and 50Hz harmonics. b) shows the frequencies recorded after the Spike2 scripts have been applied.

cosine waves. The matching sine wave is continually readjusted every 5 sinusoidal cycles to ensure that the waveform removed most accurately matches the harmonic series within the data. The resulting ECG signal is free from the unwanted harmonic series (figure 2.9b). The advantage of processing the ECG in this way is that it allows waveforms, specifically identified as undesirable, to be removed from the ECG signal without losing components of the signal which are important to the final waveform morphology. This improves confidence in fine wave amplitude measurements made when comparing ECG final waveforms.

The recorded ECG signals were processed and then converted into the 12 leads of the ECG using the methods of Einthoven (1912), Goldberger (1942) and Wilson (1934) (equations 2.4, 2.7 & 2.8, page 46). The affects of processing the ECG signal after the 12 leads had been

derived was considered, but this had no affect on ECG wave amplitudes or durations and therefore was not pursued further.

2.9.3. ECG analysis

Spike2 analysis scripts were run on each two minute data set to obtain an averaged ECG waveform of ~120 beats. The difference between two averaged waveforms (recorded in different conditions) was calculated by subtracting the waveform of the first time period from the waveform of the second time period (figure 2.10). The script repeats this process in each of the 12 ECG leads. The advantage of averaging the ECG waveform in this way is that it eliminates any effects on wave amplitude caused by the respiratory cycle (due to changing chest inflation) and provides a waveform that represents the overall trend of cardiac events during the period of ECG recording.

Figure 2.10. Averaged waveforms in two conditions from one subject

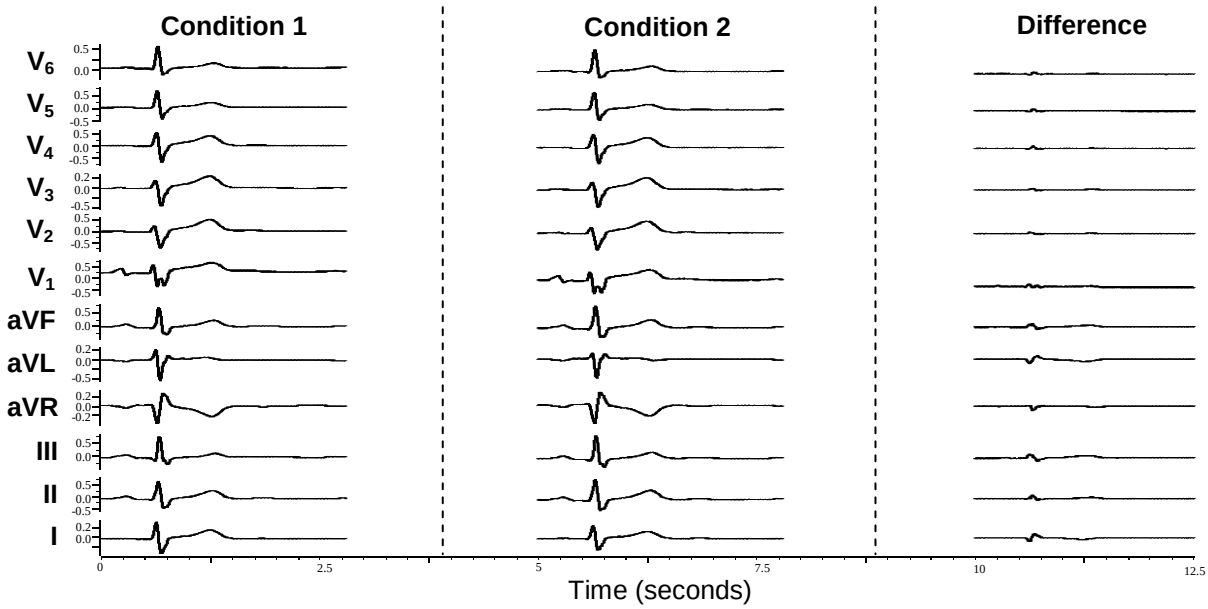


Figure 2.10. Data from subject 1 showing the averaged waveform in each lead for condition 1 and condition 2. The difference between the two conditions is shown in the third column. *i.e.,* a negative wave represents a decrease from condition 1 to condition 2.

Calculating the wave amplitudes and durations

Automated Spike2 analysis scripts were used to calculate wave amplitudes and interval durations. These scripts were programmed to identify the peaks of each wave in the order that they appear in the cardiac cycle. Double and triple differentiation of the ECG waveform was calculated and used to identify the beginning of the Q wave, the J point and the end of the T wave (for use when measuring interval durations) (figure 2.11). Every file was manually checked to ensure that each ECG wave had been correctly recognised. All wave amplitude measurements were calculated by the computer software.

Figure 2.11. Double differentiation of the ECG waveform

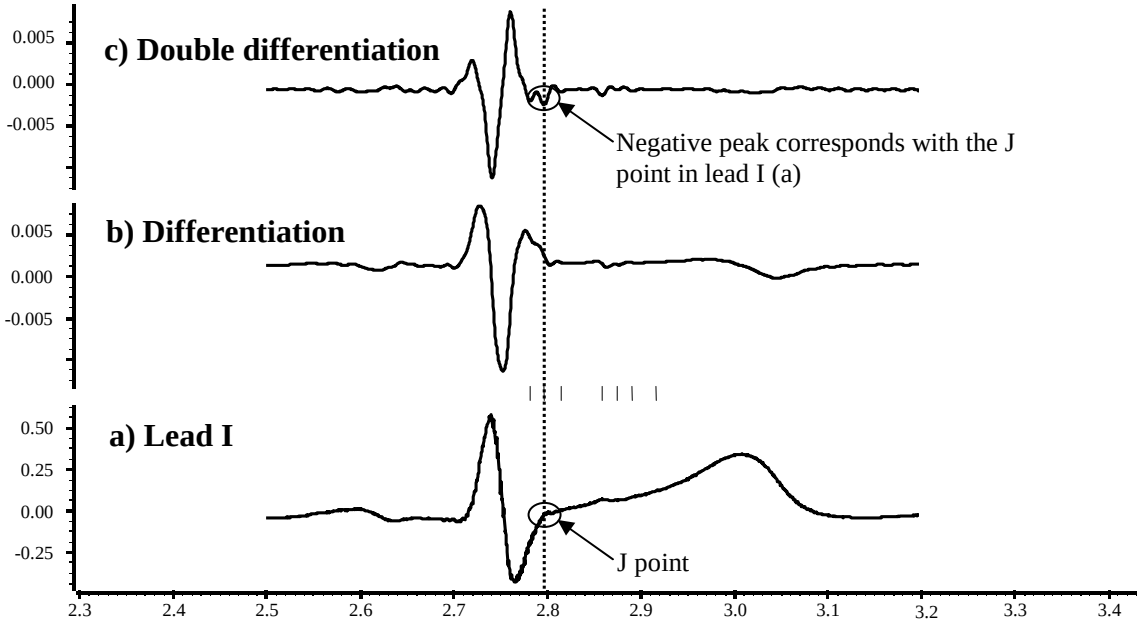


Figure 2.11. Double differentiation (c) of the ECG waveform (a) was used to calculate the points of inflection at the beginning of the Q wave, the J point (shown) and the end of the T wave.

The amplitudes of the R and T waves and the J and ST(80) points (figure 2.3, page 41) were calculated from the averaged waveforms. The Spike2 script was programmed to measure each wave height (in mV) from zero and the time at which the peaks occurred. The isoelectric line

is considered to be an electrically neutral period in the cardiac cycle and is measured from the period between the end of the P wave and the beginning of the Q wave (figure 2.3, page 41) (Braunwald *et al.*, 2001). This isoelectric line was subtracted from all wave heights to give the wave amplitude (figure 2.3, page 41).

The ST segment was calculated from the J point height and 80 milliseconds after the J point (ST [80]) (Braunwald *et al.*, 2001). The QRS axis was calculated in MS excel using equation 2.9.

Equation 2.9.

$$\text{QRS axis} = (\tan^{-1}(b/a)) + (\text{axis } \theta \text{ of } a)$$

Where (a) is the amplitude of the RS wave (R wave + S wave) in the frontal plane lead with the largest QRS complex and (b) is the height of the RS wave in the corresponding isoelectric lead (e.g. lead I is isoelectric to aVF, lead II is isoelectric to aVL and lead III is isoelectric to aVR).

(Houghton *et al.*, 2003)

The QT interval represents the duration of ventricular activation and recovery. It was measured from the beginning of the Q wave to the end of the T wave (figure 2.3, page 41). The QT interval was corrected for changes in heart rate using Bazett's formula (equation 2.10). The accuracy of this formula has been questioned when it is used for subjects with exceptionally high or low resting heart rates (Wagner, 2008; Rautaharju *et al.*, 2009). However, it was deemed appropriate for the present studies because participating subjects' heart rates were always within a normal range (57-62 bpm). Bazett's formula is also the most widely used correction for the QT interval in normal clinical practice (Kenigsberg *et al.*, 2007).

Equation 2.10.

$$\text{QTc} = \frac{QT \text{ interval}}{\sqrt{R-R \text{ interval}}} \qquad \text{(Bazett, 1920)}$$

2.9.4. Calibration

The ECG was calibrated using a patient simulator calibration box (Dynatech Nevada Inc., Model 212B, Nevada, USA) which produces an ECG signal comparable to that recorded at the limb electrodes. Standardised ECG wave amplitudes from the patient simulator box were compared to the measured wave amplitudes from the ECG apparatus used in the present study, after signal processing (table 2.1). The wave amplitudes recorded using the present apparatus accurately represented those produced by the patient simulator to within 0.02 ± 0.0mV.

Table 2.1. Actual and measured R and T wave amplitudes from the patient simulator calibration box.

Wave	ECG Lead	Actual wave amplitude	Measured wave amplitude	Difference
R wave	I	1.00mV	1.01 ± 0.0mV	0.01 ± 0.0mV
	II	1.30mV	1.30 ± 0.0mV	0.00 ± 0.0mV
	III	0.30mV	0.29 ± 0.0mV	0.01 ± 0.0mV
T wave	I	0.25mV	0.25 ± 0.0mV	0.00 ± 0.0mV
	II	0.30mV	0.32 ± 0.0mV	0.02 ± 0.0mV
	III	0.05mV	0.07 ± 0.0mV	0.02 ± 0.0mV

To confirm that measurements made in the present study were comparable with both previous studies and measurements made in a clinical setting, ECGs were recorded from each subject using the D360 ECG apparatus and a standard clinical ECG machine (Philips Hewlett Packard Pagewriter 200) without disconnecting the electrodes. ECG wavelengths from each lead in both recordings were compared in each subject. The largest difference between the R waves recorded in all leads using both apparatus was 8% (0.07 ± 0.01mV in V4, $P > 0.90$) (table

2.2). On the ECG printout from the clinical machine, this difference corresponds to 0.7mm. In the T wave, the largest discrepancy was 4% (0.04 ± 0.00mV change in Lead I, $P < 0.05$) (table 2.2) and this would represent a 0.4mm difference on a paper ECG trace. Because ECG measurements could not be made simultaneously with both machines, a small degree of error was expected. In addition, wave amplitudes recorded with the standard clinical ECG machine were measured by hand and thus were subject to small human error. A maximum disagreement of 1mm was therefore considered acceptable.

Table 2.2. Comparison of ECG wave amplitudes measured from the Phillips HP Pagewriter 200 and the ECG apparatus used in this study in 18 subjects

Wave	ECG Lead	Phillips Pagewriter 200 wave amplitude (mV)	Current ECG apparatus wave amplitude (mV)	Difference (mV)
R wave	I	0.66 ± 0.1	0.68 ± 0.1	0.02 ± 0.00
	II	1.04 ± 0.1	1.00 ± 0.1	0.04 ± 0.01
	III	0.53 ± 0.1	0.54 ± 0.1	0.01 ± 0.00
	aVR	-0.83 ± 0.1	-0.84 ± 0.1	0.01 ± 0.00
	aVL	0.25 ± 0.0	0.29 ± 0.0	0.04 ± 0.01
	aVF	0.74 ± 0.1	0.71 ± 0.1	0.03 ± 0.01
	V_1	0.24 ± 0.0	0.25 ± 0.0	0.01 ± 0.00
	V_2	0.55 ± 0.1	0.54 ± 0.1	0.01 ± 0.00
	V_3	0.82 ± 0.1	0.75 ± 0.1	0.07 ± 0.01
	V_4	1.37 ± 0.2	1.30 ± 0.1	0.07 ± 0.01
	V_5	1.52 ± 0.1	1.54 ± 0.1	0.02 ± 0.01
	V_6	1.24 ± 0.1	1.27 ± 0.1	0.03 ± 0.01
T wave	I	0.33 ± 0.0	0.37 ± 0.0	0.04 ± 0.00
	II	0.36 ± 0.0	0.39 ± 0.0	0.03 ± 0.00
	III	0.04 ± 0.0	0.03 ± 0.0	0.01 ± 0.00
	aVR	-0.34 ± 0.0	-0.36 ± 0.0	0.02 ± 0.00
	aVL	0.15 ± 0.0	0.17 ± 0.0	0.02 ± 0.00
	aVF	0.20 ± 0.0	0.20 ± 0.0	0.00 ± 0.00
	V_1	0.05 ± 0.0	0.03 ± 0.0	0.02 ± 0.01
	V_2	0.63 ± 0.1	0.63 ± 0.1	0.00 ± 0.00
	V_3	0.62 ± 0.1	0.60 ± 0.1	0.02 ± 0.01
	V_4	0.56 ± 0.1	0.56 ± 0.1	0.00 ± 0.00
	V_5	0.47 ± 0.0	0.49 ± 0.1	0.02 ± 0.01
	V_6	0.37 ± 0.0	0.40 ± 0.0	0.03 ± 0.01

2.9.5. Clinical Thresholds

Table 2.3. Summary of AHA/ACCF/HRS clinical thresholds for abnormality (non gender specific). Thresholds apply to all leads unless stated otherwise

ECG measurement	Clinical threshold - Frontal plane	Clinical threshold - Transverse plane
Wave amplitude		
ST segment height	Elevation of >0.1mV Depression of < -0.1mV	Elevation of >0.2mV in V_{2-3} Elevation of >0.1mV in V_1 & V_{4-6} Depression of < -0.05mV in V_{2-3} Depression of < -0.1mV in V_1 & V_{4-6}
T wave amplitude	>0.5mV	>1.0mV
Interval duration		
QTc interval	> 0.43s	> 0.43s
Electrical axis		
QRS axis	< > -30° to +90°	No clinical threshold

All recorded ECG measurements were quantified according to current clinical thresholds set out by the American Heart Association (AHA), the American College of Cardiology Foundation (ACCF) and the Heart Rhythm Society (HRS) (Surawicz *et al.*, 2009; Wagner *et al.*, 2009; Rautaharju *et al.*, 2009). Additional clinical guidelines on specific T amplitudes were taken from Wagner (2008). A summary of these clinical guidelines can be seen in table 2.3. The clinical thresholds presented here are for markers of ischemia within the myocardium. These were of primary interest in the experiments in this thesis investigation.

Due to the effects of gender on the ventricular repolarisation phase of the cardiac cycle, T wave amplitudes and ST segment height in the transverse plane leads were compared

collectively to the non gender specific clinical thresholds in table 2.3 and in addition, data was separated and compared to gender specific clinical thresholds summarised in table 2.4.

Table 2.4. Summary of gender specific AHA/ACCF/HRS clinical thresholds for abnormality in the transverse plane leads.

ECG measurement	Clinical thresholds - Men	Clinical thresholds - Women
T wave amplitude		
V_1	>0.65mV	>0.20mV
V_2	>1.45mV	>0.85mV
V_3	>1.35mV	>0.85mV
V_4	>1.15mV	>0.85mV
V_5	>0.90mV	>0.70mV
V_6	>0.65mV	>0.55mV
ST segment height		
V_1	Elevation of >0.10mV	Elevation of >0.10mV
V_{2-3}	Elevation of >0.25mV	Elevation of >0.15mV
V_{4-6}	Elevation of >0.10mV	Elevation of >0.10mV

2.10. Echocardiography

2.10.1. Background in echocardiography

Echocardiograms visualise the structure and function of the heart non-invasively and can be used to identify wall motion abnormalities and diastolic dysfunction associated with myocardial ischemia caused by coronary artery disease. Echocardiography was used in this thesis investigation to study the potentially ischemic effects of hypocapnia on wall motion (measured with M-mode and tissue Doppler analysis) and diastolic function (measured with Doppler blood flow analysis).

An echocardiographic machine utilizes an ultrasonic transducer placed on the chest wall and creates images which are displayed on a computer screen. Ultrasonic transducers emit waves

with a frequency of ~2,000,000 cycles per second (2 MHz) in intermittent bursts (Feigenbaum, 1981). In the interval between each burst, the transducer becomes a receiver waiting for reflected echo waves. Ultrasonic waves hit structures of different media (*e.g.* soft tissue, muscle, red blood cells) and are reflected back towards the transducer. The distance of the structure from the ultrasonic transducer is calculated (by equation 2.11) and converted into an electrical impulse which is passed through a signal amplifier and displayed as an echocardiographic image on the computer screen.

Equation 2.11.

$$\text{Distance} = \frac{\text{Velocity of sound waves}}{\text{Wave transit time}}$$

Where the velocity of sound was taken as 1540 ms^{-1} and the wave transit time represents the time it takes for a wave to be emitted from the transducer and be reflected back off a medium within the body

Figure 2.12. Echocardiographic imaging planes of the heart

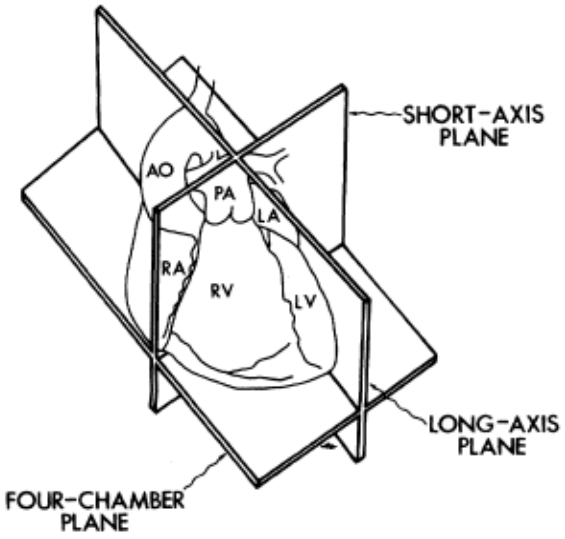


Figure 2.12. Two-dimensional echocardiographic imaging planes. RV = right ventricle, LV = left ventricle, RA = right atria LA = left atria, PA = pulmonary artery (From Henry *et al.* (1980). Report of the American Society of Echocardiography Committee on Nomenclature and Standards in Two-dimensional Echocardiography. *Circulation*, 62(2): 212)

Figure 2.13. Echocardiographic views of the heart

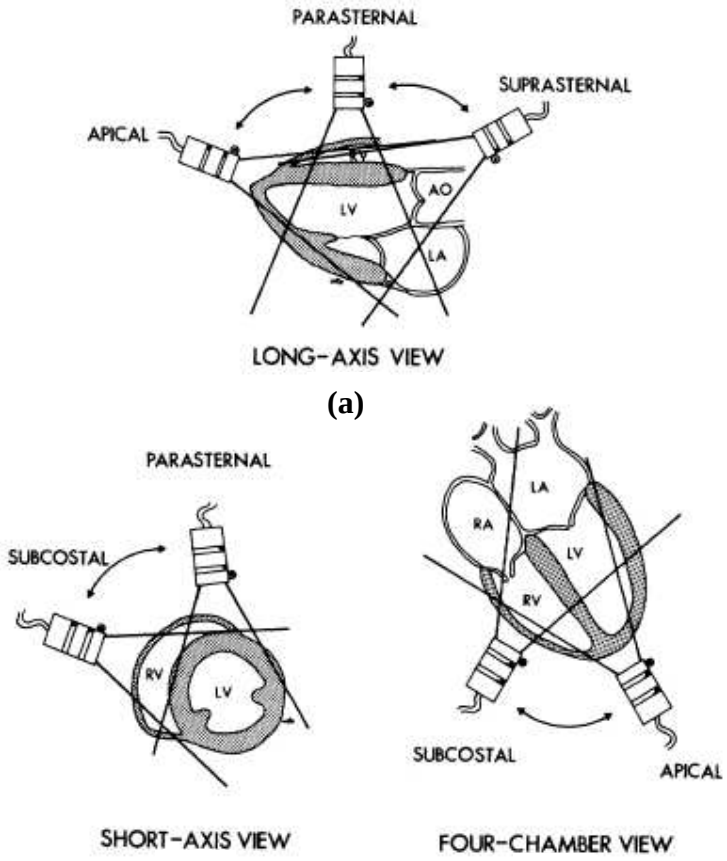


Figure 2.13. Transducer orientations of the three echocardiographic planes of the heart. RV = right ventricle, LV = left ventricle, RA = right atria LA = left atria, AO = aorta (From Henry *et al.* (1980). Report of the American Society of Echocardiography Committee on Nomenclature and Standards in Two-dimensional Echocardiography. *Circulation*, 62(2): 212)

Different modes of echocardiography require different transducer positions and these positions have been standardised to elicit consistency with recordings in both clinical and research settings (Henry *et al.*, 1980). The heart is viewed on 3 anatomical planes (long axis, short axis and four chamber plane) (figure 2.12) from 4 'viewing windows'. The long axis plane can be viewed from the apical, parasternal or suprasternal chest locations (figure 2.13a). The short axis plane can be viewed from the parasternal or subcostal locations of the chest

(Figure 2.13b). The four chamber plane can be viewed from either the apical or subcostal chest positions (figure 2.13c).

Echocardiography was used in experiments studying the effects of hypocapnia on heart function in normal subjects. All echocardiography measurements were made by a trained echocardiography technician using a Phillips SONOS 7500 ultrasound system (M2424A, Andover, Massachusetts, USA). Subjects lay recumbent, on their left side, causing the heart to move closer to the chest wall, making it easier to image. Recorded data was saved and analysed offline by an independent echocardiography technician who was blinded to the different conditions. Analysis was done using the pre-programmed analysis tools built into the echocardiographic machine software.

Three different modes of echocardiography were recorded: Two-dimensional directed M-mode, Doppler blood flow & tissue Doppler echocardiography. The methods of these modes are described below.

2.10.2. M-mode echocardiography

Two-dimensional directed M-mode echocardiography depicts the motion of the left ventricle walls (calculated by the change in distance of object in relation to the transducer) over time (Feigenbaum, 1981). M-mode images have no resemblance to a specific anatomical structure but depict a pattern of motion from the target myocardial structure throughout the cardiac cycle (Arvan, 1984). M-mode echocardiography allows for more subtle changes in wall and valve motion to be studied.

Figure 2.14. M-mode echocardiogram of the left ventricle.

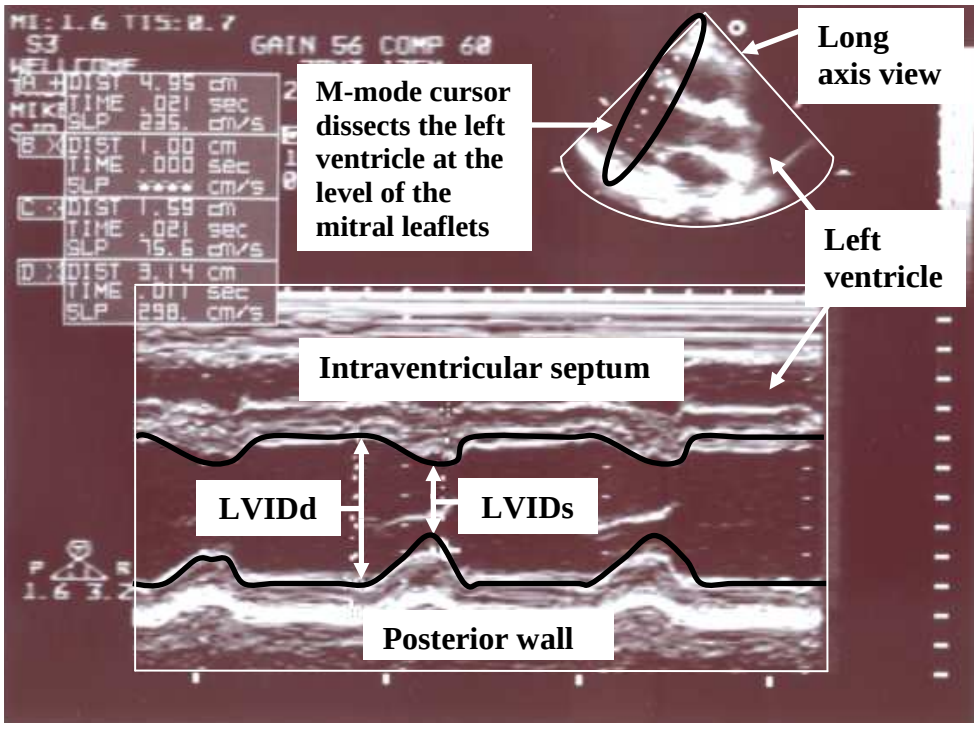


Figure 2.14. Transmitral M-mode echocardiogram. Measures left ventricle intraventricular diameter in diastole (LVIDd) and systole (LVIDs).

In this thesis investigation, M-mode echocardiographs were recorded from the parasternal long axis view (figure 2.13a). The area of the heart to be imaged was determined from an M-mode cursor which is placed on the target location of a two-dimensional long axis echocardiographic image (figure 2.14). In this case, the cursor was placed at the level of the tips of the mitral valve, dissecting the left ventricle. From this position, movement of the intraventricular septum and left ventricular posterior wall can be recorded (figure 2.14). Wall motion in the left ventricle was determined by measuring the distance between the intraventicular septum and the posterior wall (LVID) and comparing this difference between diastole (LVIDd) and systole (LVIDs) (figure 2.14). Fractional shortening (FS) is a measure of degree to which the left ventricle gets smaller during myocardial contraction. It was calculated from the LVID using equation 2.12.

Equation 2.12.

$$FS = \left(\frac{LVIDd - LVIDs}{LVIDd} \right) \times 100$$

Two-dimensional M-mode echocardiographic measurements were made in both baseline and hypocapnic conditions during each trial. LVID was compared in diastole and systole to allow estimation of the degree of wall motion during myocardial contraction. Normal mean values for LVID during diastole are 4.6 ± 0.5cm and 2.9 ± 0.5cm during systole (1.7 ± 0.5cm difference) (table 2.5) (Salcedo, 1978). Normal mean values for FS are 37 ± 7% and any reduction below 25% is considered clinically significant (Weyman, 1982). During myocardial ischemia, the LVID difference and FS will decrease as wall motion becomes inhibited in the ischemic region (Braunwald *et al.*, 2001).

Table 2.5. Normal clinical values for echocardiographic measurements made in M-mode, Doppler blood flow mode and tissue Doppler mode.

Measurement	**Normal values**
M-mode echocardiography	
LVIDd	4.6 ± 0.5cm
LVIDs	2.9 ± 0.5cm
Fractional Shortening	>25% (mean 37 ± 7%)
Doppler echocardiography	
E/A ratio	>1
Deceleration time	<220ms (mean 200 ± 40ms)
Tissue Doppler echocardiography	
Septal wall velocity (diastole)	11.4 ± 2.6cm/s
Septal wall velocity (systole)	7.1 ± 1cm/s
Lateral wall velocity (diastole)	15.4 ± 3.9cm/s
Lateral wall velocity (systole)	10.0 ± 2.2cm/s

2.10.3. Doppler echocardiography

Doppler echocardiography measures the velocity of blood flow into the left ventricle. It is based on the principles of the 'Doppler effect'. Changes in the frequency of sound waves as a result of movement of the source relative to the target are known as Doppler shifts (Arvan, 1984). As the source moves towards a stationary target, the frequency of sound waves becomes larger; as the source moves away from the target, the frequency becomes smaller. In the case of Doppler echocardiography, the source is stationary (echocardiographic transducer) and the target is moving (red blood cells) (Arvan, 1984). Ultrasonic waves are emitted from the transducer at a known frequency. If these waves strike a target that is stationary, they are reflected back at the same frequency. If the target is moving, such as red blood cells, waves are reflected back to the transducer at an alternative frequency which is dependent on the direction of blood flow. When blood flow is moving towards the echocardiographic transducer, the frequency of reflected ultrasonic waves is increased and when blood flow moves away from the transducer, the frequency of reflected ultrasonic waves is attenuated (Arvan, 1984). The change in frequency between the emitted and returning waves is known as the Doppler shift and is calculated by the echocardiographic machine. These changes in frequency can be used to calculate the maximum velocity of blood flow that is directly parallel to the axis of the ultrasonic beam (equation 2.13) (Baker *et al.*, 1977).

Equation 2.13.

$$\text{Velocity of blood flow} = \frac{\Delta f C}{2 f \cos\theta}$$

Where Δf = change in frequency, C = velocity of sound in tissue (1540 ms^{-1}), f = frequency of waves emitted by the transducer and θ = angle of the transducer in relation to blood flow (assumed to be 0˚).

(Braunwald *et al.*, 2001)

The pulse repetition frequency represents the sampling rate of the reflected ultrasonic waves (Baker *et al.*, 1977). To prevent aliasing, the pulse repetition frequency must be at least twice the expected Doppler frequency shift (Nyquist, 2002). A large frequency shift can occur when measuring blood flow due to its high velocity (10-100cm/s) (Erbel *et al.*, 1996). Therefore, the pulse repetition frequency is set as high as is possible for the depth of the sample volume (if both the depth of the sample volume and the pulse repetition frequency are high, there will not be enough time for ultrasonic waves to be reflected from the sample before the next burst is emitted). The sensitivity of the transducer is increased to detect blood flow signals of low amplitude and a high pass filter is applied to the data to remove signals of low velocity, originating from other moving tissues (Garcia *et al.*, 1998).

Figure 2.15. Doppler blood flow echocardiogram

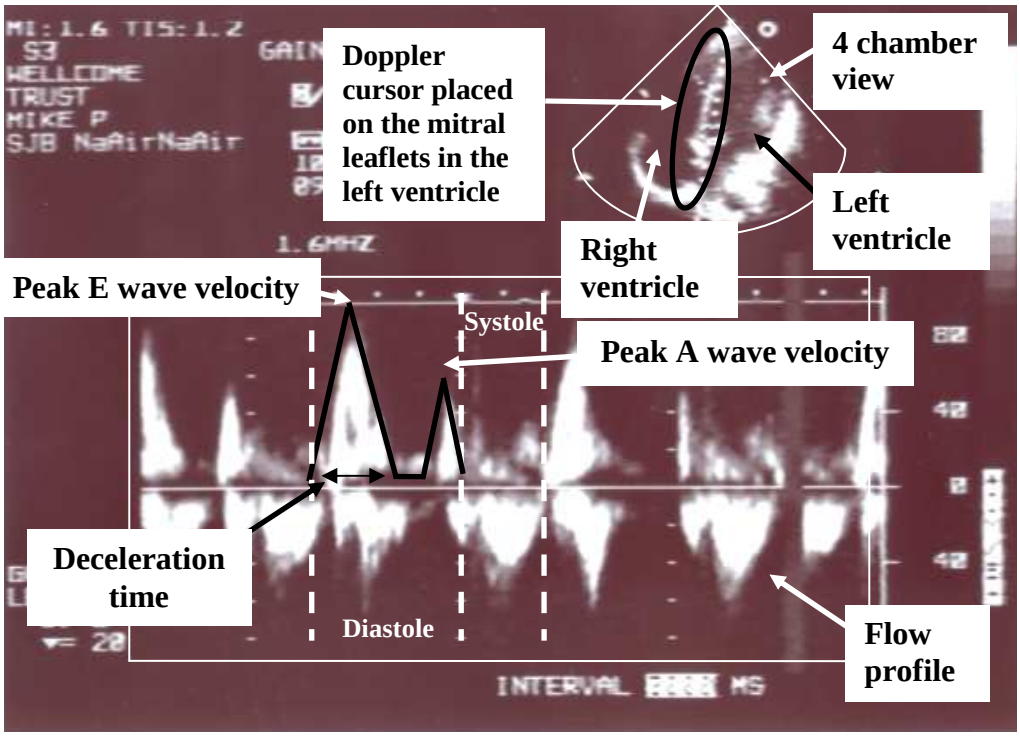


Figure 2.15. Spectral mode flow profile from subject 1, showing left ventricle filling patterns. E wave = early diastole. A wave = late diastole. Transducer placed in the apical window showing the 4-chamber plane

In the present investigation, Doppler echocardiographs were recorded from the apical position showing the 4 chamber view (figure 2.13c, page 63). The Doppler cursor was placed at the tips of the mitral leaflets. This records the flow of blood from the left atrium, across the mitral valve and into the left ventricle. Doppler measurements were recorded for several cardiac cycles and displayed in spectral mode as a flow profile (figure 2.15). Spectral mode depicts increases in reflected wave frequency (blood flow towards the transducer) as positive deflections on the flow profile and decreases in wave frequency (blood flow away from the transducer) as negative deflections. The height of these deflections is relative to the blood flow velocity.

On a Doppler trace, blood flow across the mitral valve during early diastole is represented by the E wave (figure 2.15). Peak blood flow velocity during early diastole is represented by the peak of the E wave. As the left ventricle fills with blood, the pressure gradient between the left ventricle & left atrium decreases, causing the flow of blood into the left ventricle to fall. This is measured by the deceleration time (figure 2.15). Blood flow into the left ventricle is then increased by atrial contraction during late diastole causing the A wave (figure 2.15). Peak blood flow velocity during late diastole is represented by the peak of the A wave.

Doppler blood flow echocardiographic measurements were recorded in normocapnia and hypocapnia and the E/A (E wave/A wave) ratio and deceleration time were calculated. In normal healthy subjects, atrial contraction accounts for only 30% of total diastolic filling. During this period, blood flow velocity is low. Thus the A wave is always smaller than the E wave (Hamlin *et al.*, 2004). The difference between these waves is represented by the E/A ratio and in normal subjects this is always greater than 1 (Hamlin *et al.*, 2004) (table 2.5).

Deceleration time is always short due to the fast inflow of blood into the left ventricle during early diastole (~200 ± 40ms) (Nishimura & Tajik, 1997). Myocardial ischemia causes the relaxation phase of the cardiac cycle to be impaired resulting in Grade I (mild) diastolic dysfunction (Nishimura & Tajik, 1997). Therefore, if hypocapnia was to cause myocardial ischemia in the present study, blood flow into the left ventricle during early diastole would be reduced. This would cause a decrease in E wave amplitude and a compensatory increase in A wave amplitude, resulting in a decreased E/A ratio (<1) and prolonged deceleration time (>0.22 seconds) (Garcia *et al.*, 1998).

2.10.4. Tissue Doppler echocardiography

Tissue Doppler echocardiography measures the velocity of left ventricular wall motion during diastole and systole. Tissue Doppler echocardiography uses the same principles as Doppler blood flow echocardiography. The myocardium moves at low velocity (<10 cm/s) and reflects ultrasonic waves of high amplitude (Erbel *et al.*, 1996). To view Doppler frequency shifts from the tissues, the high pass filter used in standard Doppler echocardiography is removed and the sensitivity of the ultrasonic transducer is reduced so that low amplitude signals (from blood flow) are not recorded (Mcdicken *et al.*, 1992). Because the expected Doppler frequency shifts are small, the pulse repetition frequency can be reduced allowing a sample volume of increased depth. As with standard Doppler measurements, a sample volume is positioned over the myocardial area of interest on the 4 chamber plane and a Doppler cursor is placed on the myocardial wall of interest (figure 2.16, 2.17). Tissue Doppler wall velocity profiles are displayed in spectral mode (figure 2.16, 2.17). The height of the waves on these profiles represents the velocity of the target wall at that point in the cardiac cycle.

Figure 2.16. Tissue Doppler echocardiogram of the septum.

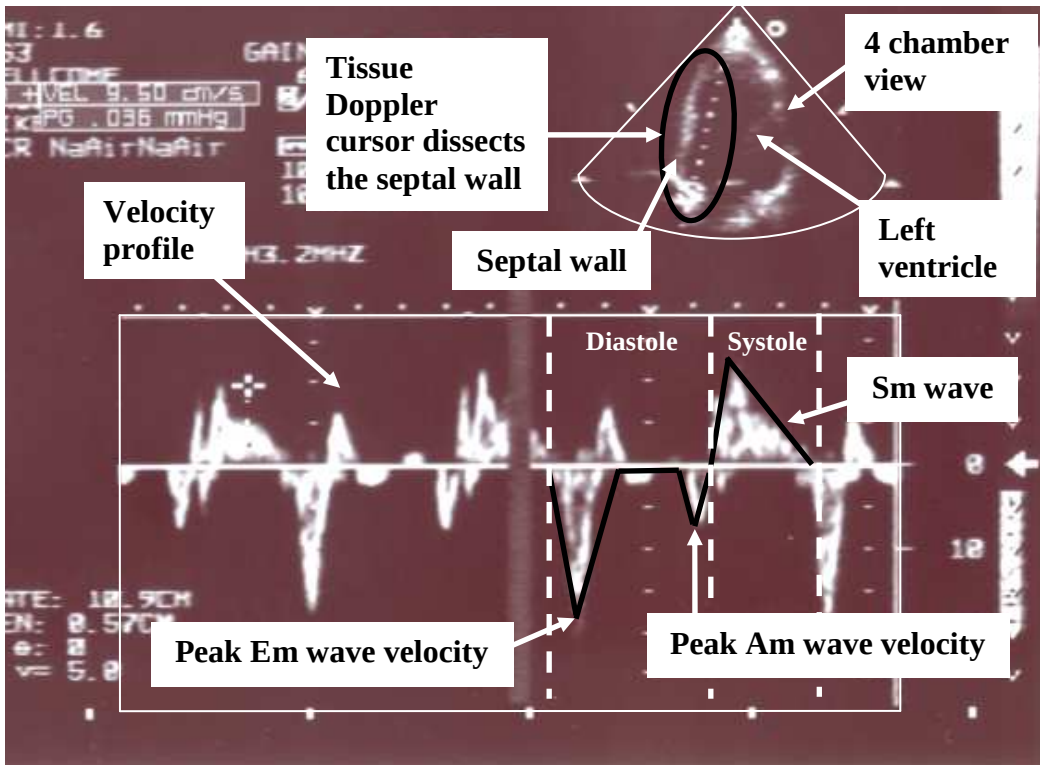


Figure 2.16. Spectral mode velocity profile from subject 1, showing velocity of the septum. Em wave = early diastole. Am wave = late diastole. Sm wave = systole. Transducer placed in the apical window showing the 4-chamber plane

For tissue Doppler recordings made in the present study, the echocardiographic transducer was placed in the apical position showing the 4 chamber view (figure 2.13c, page 63). The Doppler cursor was placed on the septum (figure 2.16) and lateral wall (figure 2.17) segments of the left ventricle. Tissue Doppler imaging depicts myocardial wall velocities as Em waves (early diastole), Am waves (late diastole) and Sm waves (systole) which allows wall velocities to be distinguished at different stages of the cardiac cycle (figures 2.16, 2.17). The peaks of these waves represent the peak velocity of the myocardial wall being imaged.

Figure 2.17. Tissue Doppler echocardiogram of the lateral wall.

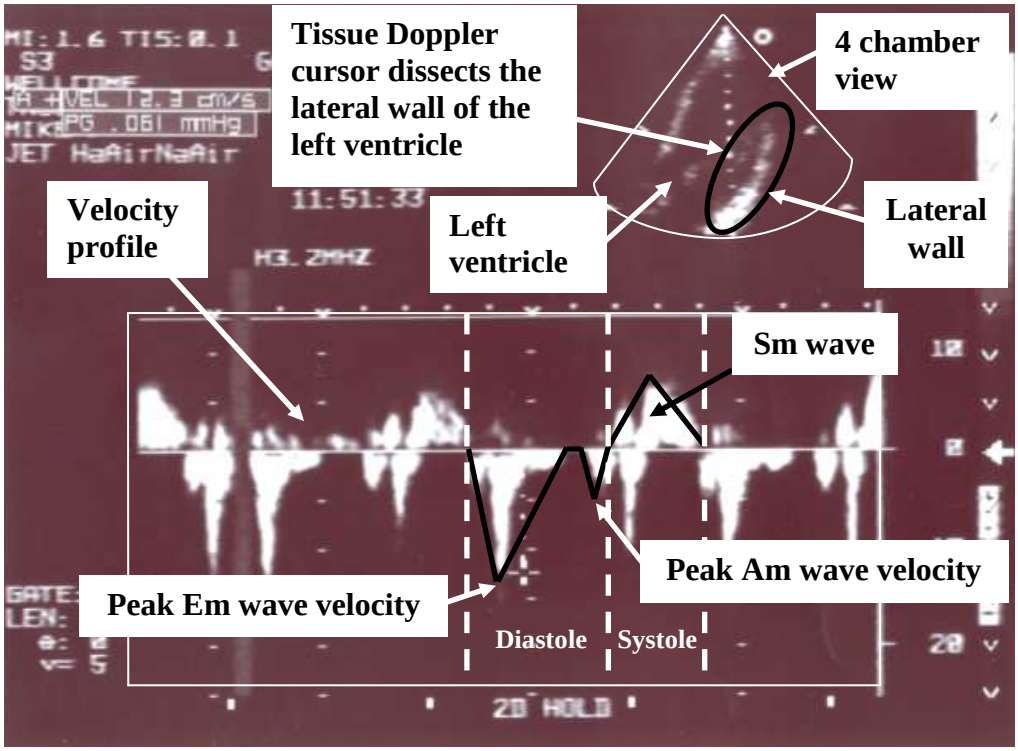


Figure 2.17. Spectral mode velocity profile from subject 1, showing velocity of the lateral wall. Em wave = early diastole. Am wave = late diastole. Sm wave = systole. Transducer placed in the apical window showing the 4-chamber plane

Normal mean values for myocardial wall velocities are presented in table 2.5. During myocardial ischemia, wall velocities are significantly reduced by 31-57% in diastole (Em wave) and 39% in systole (Sm wave) and the decrease in wall velocity is correlated with the size of the ischemic region (Garcia-Fernandez *et al.*, 1999; Kostkiewicz *et al.*, 2003). In the present study, a decrease in diastolic wall velocity below 8cm/s during hypocapnia would be suggestive of the presence of myocardial ischemia (Garcia-Fernandez *et al.*, 1999; Garcia *et al.*, 1998; Kostkiewicz *et al.*, 2003).

Chapter 3

Does ECG electrode placement modification cause clinically significant changes in the standard 12 lead ECG of healthy subjects at rest?

3.1. Summary

In experiments studying the effects of hypocapnia, induced by mechanical hyperventilation, on the ECG, Rutherford *et al.,* (2005) placed the limb electrodes on the torso, as is routinely done during exercise stress testing or when the limbs are inaccessible. It is unclear to what degree such electrode modification alters the amplitudes of the ECG waves, or whether it produces clinically important, false positive ECG changes in healthy subjects (*e.g.* ST segment changes of greater than 0.1mV, T wave changes greater than 0.5mV in the frontal plane or 1mV in transverse plane, QRS axis shifts or alterations to the QTc interval).

This experiment demonstrated in healthy and semi-recumbent subjects (n = 18), that electrode modification caused small R and T wave amplitude changes in the frontal plane and QRS axis changes that are statistically, but not clinically significant. For example, in lead I, electrode modification caused a decrease in the amplitude of the R wave of 21% (0.13mV, $P < 0.001$) and T wave of 19% (0.06mV, $P < 0.001$). In the transverse plane, modification does not produce statistically or clinically significant changes in ECG wave amplitudes or in ST segment morphology or QTc interval duration. When separated by gender, T wave amplitude and ST segment height in the transverse plane remain unaffected by electrode modification.

This study shows that electrode modification does significantly alter the amplitude of the R and T wave. However, these ECG changes are not clinically significant in healthy subjects and thus have no effect on the clinical specificity of the 12 lead ECG.

3.2. Introduction

The position of the ECG electrodes was defined by Einthoven (1912) (limb electrodes) and Barnes *et al.,* (1938) (precordial electrodes). Motion of the limbs can disrupt the ECG sufficiently to make the limb electrode placement impractical during exercise testing. Mason and Likar (1966) developed an alternative electrode placement to avoid this muscular interference during exercise. They repositioned the arm electrodes onto the "infraclavicular fossa medial to the border of the deltoid muscle" and the left leg electrode onto "anterior axillary line, halfway between the costal margin and the crest of the illium" (Mason & Likar, 1966) (Modified[1], table 3.1). It was claimed that ECGs recorded using this electrode modification vary only slightly from the standard electrode placement, but data presented showed an increase in R and T wave amplitude of up to 40% in leads I-III (table 3.2). Subsequent studies have disputed the accuracy of the M-L modified electrode placement claiming it could result in false diagnosis of disease in normal healthy patients because the modified placement shifts wave amplitudes and electrical (QRS) axes beyond normal limits (Kleiner *et al.,* 1978; Rautaharju *et al.,* 1980; Gamble *et al.,* 1984; Papouchado *et al.,* 1987; Edenbrandt *et al.,* 1989; Sevilla *et al.,* 1989).

The modified electrode placement was of interest in this thesis investigation because Rutherford *et al.,* (2005) used a modified electrode placement when studying the effects of hypocapnia on the ECG. A review of the literature revealed much disagreement as to the

Table 3.1. Alternative electrode placements of the standard 12 lead ECG. Standard electrode placement indicated by blue crosses on the limbs.

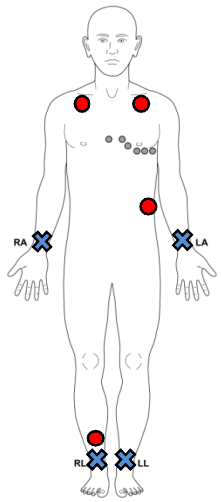
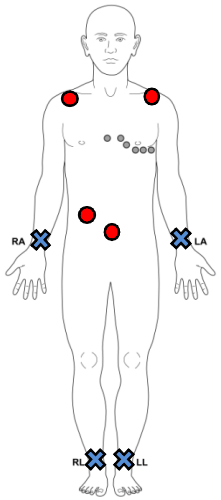
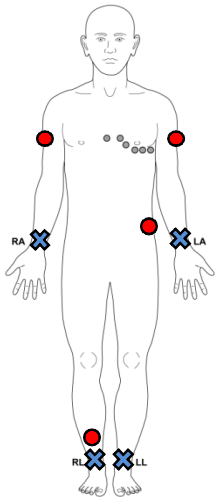
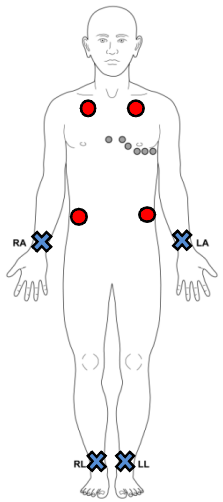
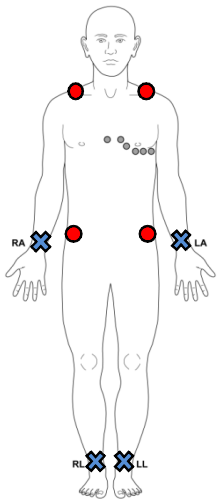
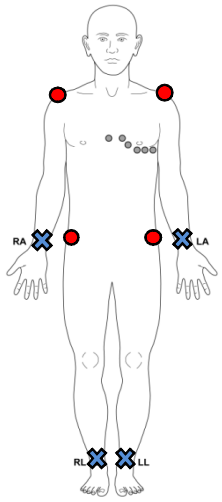
Author	Electrode placement	Diagram	Author	Electrode placement	Diagram	Author	Electrode placement	Diagram
Mason & Likar (1966)	**M-L (Modified (Modified[1])** Arm electrodes are placed on the infraclavicular fossa medial to the border of the deltoid muscle. The left leg electrode is placed on the anterior axillary line, halfway between the costal margin and the crest of the illium		Diamond *et al.,* (1979)	**Diamond (Modified[2])** Arm electrodes are placed below the outer third of the right/left cavicle. The left leg electrode is placed 4cm below the umbilicus and the right leg electrode is positioned in the mid clavicular line at the level of the umbilicus		Edenbrandt *et al.,* (1989)	**Lund (Modified[3])** Arm electrodes are placed on the lateral side of the arms at the level of the axillary fold. The left leg electrode is positioned on the left iliac crest in the anterior axillary line	
Sevilla et al., (1989)	**Sevilla (Modified[4])** Arm electrodes are placed on the right & left mid-clavicular lines, just below the clavicle. The leg electrodes are placed on the left & right anterior axillary lines, just above the subcostal margins		Krucoff *et al.,* (1994)	**Krucoff (Modified[5])** Arm electrodes are placed on the lateral extent of the clavicle. The leg electrodes are placed on the left & right iliac crests in the midaxillary line		Takuma *et al.,* (1995)	**Takuma (Modified[6])** Arm electrodes are placed on the left & right anterior acromial regions. The leg electrodes are placed on the left & right anterior superior iliac spines	

Table 3.2. Frontal plane leads. Table shows the absence of quantifiable data included in previous studies investigating the effects of modified electrode placements on the ECG.

Table 3.2.			Frontal plane leads		
Rank	**Author**	**Electrode placement/ number of subjects**	**Mean R wave change (mV) with modification**	**Mean T wave change (mV) with modification**	**Mean ST segment change height/slope (mV or mV/ms)**
1	Edenbrandt *et al.,* (1989)	**Modified[3]** (n = 10)	I: – **0.03** * II: + **0.11** * III: + **0.06** * aVR: + **0.04** * aVL: – **0.07** * aVF: + **0.10** *	No data	No data
2	Mason & Likar (1966)	**Modified[1]** (n = 19)	I: only % given* II: only % given* III: only % given*	I: only % given* II: only % given* III: only % given*	No data
3	Gamble *et al.,* (1984)	**Modified[1]** (n = 104)	I: – **0.12** * II: + **0.06** * III: + **0.21** ** aVR: no data aVL: – **0.11** * aVF: + **0.08** *	I: – **0.02** * II: no data III: no data aVR: no data aVL: no data aVF: + **0.05** *	No data
4	Rautaharju, *et al.,* (1980)	**Modified[1]** (n = 68)	I: – **0.12** * II: + **0.31** * III: + **0.24** ** aVR: no data aVL: – **0.21** * aVF: + **0.33** **	I: no data II: + **0.09** * III: + **0.07** *** aVR: no data aVL: – **0.04** * aVF: + **0.09** **	I: no data II: + **0.15 mV/ms** * III: + **0.16 mV/ms** *** aVR: no data aVL: – **0.08 mV/ms** * aVF: + **0.16 mV/ms** ***
5	Papouchado *et al.,* (1987)	**Modified[1]** (n = 29)	I: – **0.03** * II: + **0.05** ** III: + **0.07** *** aVR: no data aVL: – **0.03** ** aVF: + **0.07** ***	No data	No data
6	Kleiner, *et al.,* (1978)	**Modified[1]** (n = 75)	No data	No data	No data
7	Diamond, *et al.,* (1979)	**Modified[2]** (n = 11)	No data	No data	No data
8	Sevilla *et al.,* (1989)	**Modified[4]** (n = 44)	No data	No data	No data
9	Pahlm *et al.,* (1992)	**Modified[3/5]** (n = 26)	No data	No data	No data
10	Krucoff *et al.,* (1994)	**Modified[5]** (n = 30)	No data	No data	Mean differences in all leads **<0.02mV** *
11	Takuma *et al.,* (1995)	**Modified[6]** (n = 10)	No mean values	No mean values	No mean values
12	Jowett *et al.,* (2005)	**Modified[6]** (n = 50)	No data	No data	No data

Table 3.2. Modified[1] = M-L placement, modified[2] = Diamond placement, modified[3] = Lund placement, modified[4] = Sevilla placement, modified[5] = Krucoff placement, modified[6] = Takuma placement. Values represent mean changes from standard ECG in mV. Data is ranked with the study showing the largest ECG changes (in mV) first. * = <50% change, ** = >50% change, * = >100% change from the standard ECG.**

correct use of the modified electrode placement during exercise and at rest. A study of the specific changes caused by electrode modification was therefore conducted with particular interest placed on how modification affects R, T, and ST segment amplitudes and QRS axes and whether these changes affect the clinical specificity of the 12 lead ECG.

Table 3.3. Transverse plane leads. Table shows the absence quantifiable data included in previous studies investigating the effects of modified electrode placements on the ECG.

Table 3.3.			**Transverse plane leads**		
Rank	**Author**	**Electrode placement/ number of subjects**	**Mean R wave change (mV) with modification**	**Mean T wave change (mV) with modification**	**Mean ST segment change height/slope (mV or mV/ms)**
1	Edenbrandt *et al.,* (1989)	**Modified**[3] (n = 10)	V_1: **0.00** * V_2: – **0.02** * V_3: – **0.04** * V_4: – **0.02** * V_5: **0.00** *	No data	No data
2	Mason & Likar (1966)	**Modified**[1] (n = 19)	V_1: no data V_2: no data V_3: only % given* V_4: only % given* V_5: only % given* V_6: only % given*	V_1: no data V_2: no data V_3: only % given* V_4: only % given* V_5: only % given* V_6: only % given*	No data
3	Rautaharju, *et al.,* (1980)	**Modified**[1] (n = 68)	V_1: – **0.01** * V_2: – **0.02** * V_3: no data V_4: no data V_5: no data V_6: no data	V_1: – **0.01** * V_2: no data V_3: no data V_4: no data V_5: no data V_6: no data	No data
4	Kleiner, *et al.,* (1978)	**Modified**[1] (n = 75)	No data	No data	No data
5	Diamond, *et al.,* (1979)	**Modified**[2] (n = 11)	No data	No data	No data
6	Gamble *et al.,* (1984)	**Modified**[1] (n = 104)	No data	No data	No data
7	Papouchado *et al.,* (1987)	**Modified**[1] (n = 29)	No data	No data	No data
8	Sevilla *et al.,* (1989)	**Modified**[4] (n = 44)	No data	No data	No data
9	Pahlm *et al.,* (1992)	**Modified**[3/5] (n = 26)	No data	No data	No data
10	Krucoff *et al.,* (1994)	**Modified**[5] (n = 30)	No data	No data	Mean differences in all leads <**0.02mV** *
11	Takuma *et al.,* (1995)	**Modified**[6] (n = 10)	No mean values	No mean values	No mean values
12	Jowett *et al.,* (2005)	**Modified**[6] (n = 50)	No data	No data	No data

Table 3.3. Modified[1] = M-L placement, modified[2] = Diamond placement, modified[3] = Lund placement, modified[4] = Sevilla placement, modified[5] = Krucoff placement, modified[6] = Takuma placement. Values represent mean changes from standard ECG in mV. Data is ranked with the study showing the largest ECG changes (in mV) first. * = <50% change, ** = >50% change, * = >100% change from the standard ECG.**

Many electrode placements have been proposed claiming to eliminate discrepancies between standard and modified ECGs (table 3.1). Such modification should not cause considerable alterations to the ECG waveform in the transverse plane leads, because the modified electrode placement does not involve movement of the precordial exploring electrodes (V_{1-6}). The only

aspect of the transverse plane leads that should alter is the reference central terminal, movement of which is likely to have less effect on the final waveform amplitudes (table 3.3). Modification does, however, produce differences in wave amplitude in the frontal plane leads of the ECG (table 3.2). Most changes are no greater than +/- 50% of the standard values although it has been suggested that even these changes could result in false positive exercise stress tests in healthy subjects (Gamble *et al.*, 1984; Jowett *et al.*, 2005; Kleiner *et al.*, 1978; Pahlm *et al.*, 1992; Papouchado *et al.*, 1987; Sevilla *et al.*, 1989; Wiens & Chaitman, 1997).

The modified electrode placement has been shown to cause changes in ST segment height of between 0.02mV (Krucoff *et al.*, 1994) to 0.13 mV (Takuma *et al.*, 1995). Others claim that the modified electrode placement causes clinically important ST segment changes, despite a lack of supporting data (table 3.2 and 3.3) (Rautaharju *et al.*, 1980; Gamble *et al.*, 1984; Jowett *et al.*, 2005; Wiens & Chaitman, 1997). The extent to which the modified electrode placement affects the ST segment in the 12 lead ECG is of interest because it contains important diagnostic information for myocardial ischemia and infarction.

The modified electrode placement is thought to cause a rightward shift in the QRS axis. Data shows that the M-L placement (modified[1]) alters the QRS axis from between -3° to 45° (table 3.4) (Gamble *et al.*, 1984; Kleiner *et al.*, 1978; Papouchado *et al.*, 1987; Rautaharju *et al.*, 1980).

Table 3.4 QRS axis. Table shows the modified electrode placement used in previous studies causes a rightward shift on the frontal plane of the ECG, ranging from 4° to 48°.

Rank	Author	Electrode placement/ number of subjects	Mean QRS axis change with modification	Mean T axis change with modification
1	Sevilla *et al.,* (1989)	**Modified**[4] (n = 44)	+ **48°**	No data
2	Kleiner, *et al.,* (1978)	**Modified**[1] (n = 75)	**+ 45°**	No data
3	Papouchado *et al.,* (1987)	**Modified**[1] (n = 29)	+ **30°**	No data
4	Jowett *et al.,* (2005)	**Modified**[6] (n = 50)	+ **27°**	+ **25°**
5	Rautaharju, *et al.,* (1980)	**Modified**[1] (n = 68)	**+ 16°**	No data
6	Takuma *et al.,* (1995)	**Modified**[6] (n = 10)	+ **8°**	No data
7	Edenbrandt *et al.,* (1989)	**Modified**[3] (n = 10)	**+ 4°**	No data
8	Gamble *et al.,* (1984)	**Modified**[1] (n = 104)	− **3°**	No data
9	Pahlm *et al.,* (1992)	**Modified**[3/5] (n = 26)	No mean data	No data
10	Mason & Likar (1966)	**Modified**[1] (n = 19)	No data	No data
11	Diamond, *et al.,* (1979)	**Modified**[2] (n = 11)	No data	No data
12	Krucoff *et al.,* (1994)	**Modified**[5] (n = 30)	No data	No data

Table 3.4. Modified[1] = M-L placement, modified[2] = Diamond placement, modified[3] = Lund placement, modified[4] = Sevilla placement, modified[5] = Krucoff placement, modified[6] = Takuma placement. Modified lead placements are defined in table 3.1. Values represent mean changes from standard ECG in degrees. Data is ranked with the study showing the largest ECG changes (in mV) first.

It appears that there is no agreement on precisely which modified configuration should be used, what ECG amplitude changes are induced and whether such changes are clinically relevant. Thus, Kligfield *et al*., (2007) simply concludes that;

> "*ECGs recorded with torso placement of the extremity electrodes cannot be considered equivalent to standard ECGs for all purposes and should not be used interchangeably with standard ECGs for serial comparison*".

Rather than just measuring again whether modification produces statistically significant changes in healthy subjects, here the aim was to establish whether modification moves the

waveforms beyond the clinical limits of normality, as recently defined by the American Heart Association, the American College of Cardiology Foundation and the Heart Rhythm Society (Rautaharju *et al.*, 2009; Surawicz *et al.*, 2009; Wagner *et al.*, 2009) and Wagner (2008) (table 2.3, page 60).

Wave amplitudes, ST segment height, QRS axes and QTc interval duration were compared in both standard and modified electrode placements. By quantifying observed changes in the context of current clinical guidelines, it was hoped the clinical specificity of the modified ECG for detecting myocardial ischemia could be established. In additional analysis, T wave amplitudes and ST segment heights were separated by gender and compared to gender specific ECG clinical thresholds, as recommended by Wagner *et al.,* (2009). A secondary aim of this study was to determine whether Rutherford *et al.,* (2005) were correct in using a modified electrode placement when studying the effects of mechanical hyperventilation on the ECG. Therefore, the effect of electrode modification during mechanical hyperventilation in normocapnia was also examined.

Since the purpose of modification is to keep the limb electrodes off the extremities, they should ideally be placed just on the torso, but as near to the limbs as possible. Of all the possible modifications, that of Takuma *et al.,* (1995) (Modified[6], table 3.1) was chosen because it is nearest to this ideal and should therefore have the least effect on the ECG waveform (Gamble *et al.,* 1984; Wilson *et al.*, 1934). Arm electrodes were placed on the "anterior acromial region" and leg electrodes on the "anterior superior iliac spine" (Takuma *et al.*, 1995). This electrode placement is considered useful in a number of clinical settings because in addition to reducing the artefact of motion on the patient's ECG, it also to allows

recordings to be made without the removal of patient's shoes, trousers or stockings, eliciting faster application (important in an emergency).

3.3. Methods

Eighteen normal healthy subjects aged 24 ± 3 years old (20-30 years old) (13 male) gave informed consent to participate in the study and all experiments were approved by the Walsall Local Research Ethics Committee. Data were collected on four separate occasions and subjects lay resting and semi-recumbent on a bed. Ten Blue sensor ECG electrodes (Ambu) were placed in standard positions (figure 2.4, page 43) and four more in the modified positions (Modified[6], table 3.1). Two minute recordings were made in the standard electrode positions followed by the modified positions using the ECG apparatus previously described (chapter 2.9.2, page 50). Data from the ECG electrodes were recorded simultaneously using Spike2 data acquisition software and subsequently analysed offline.

An average waveform (of ~120 beats) was calculated in each lead for both standard and modified electrode placements. Amplitudes of the R and T waves were calculated from these waveforms. ST segment height, QRS axes and QTc interval duration were measured as previously described (chapter 2.9.3, page 55). T wave, ST segment and QTc interval data were measured because they are most commonly used as indicators of myocardial ischemia and therefore were of interest to this thesis investigation. QRS axis data was included in this study because it has been commonly used in a number of previous studies to demonstrate the extent of electrode modification on the ECG (Gamble *et al.*, 1984; Sevilla *et al.*, 1989; Jowett *et al.*, 2005; Takuma *et al.*, 1995; Edenbrandt *et al.*, 1989; Kleiner *et al.*, 1978; Papouchado *et al.*, 1987; Rautaharju *et al.*, 1980).

Wave amplitudes were defined as the wave heights (from zero) minus the isoelectric line (figure 2.3, page 41). An average of these wave amplitudes, electrical axes and interval durations was calculated for each subject over their 4 visits. T wave amplitudes and ST segment heights in the transverse plane were compared to non gender specific clinical thresholds for normality. In addition, data were also separated by gender and compared to gender specific thresholds. T wave and ST segment heights were analysed in this way because gender differences in the ventricular repolarisation phase of the cardiac cycle are known to exist (Bidoggia *et al.*, 2000; Macfarlane, 2001; Surawicz & Parikh, 2002). Recent clinical guidelines suggest that they should therefore be considered separately in leads exhibiting the largest T wave and ST segments (*i.e.*, transverse plane leads) (Rautaharju *et al.*, 2009; Wagner *et al.*, 2009).

The effects of electrode modification on the ECG during mechanical hyperventilation (in normocapnia) were also considered in nine subjects. Mechanical hyperventilation was performed as previously described (chapter 2.3, page 26). This was investigated to establish whether changes that occur at rest also occur at increased breathing rates during mechanical hyperventilation. It was not the aim of this study to examine whether the modified electrode placement affects the ECG waveform during hypocapnia.

All data presented are expressed as means ± standard error. A 2-tail paired t-test was performed in each lead to compare wave amplitudes and interval durations in the standard and modified electrode placements. The Bonferroni correction for multiple (12) comparisons was applied, on the assumption that all 12 measurements were independent and uncorrelated.

3.4. Results

As expected in transverse plane leads, figure 3.1 shows that modification of the ECG electrode placement causes no significant changes in the amplitudes of the R wave and T waves. Figure 3.2 shows this is also the case during mechanical hyperventilation in normocapnia.

In the frontal plane leads, figure 3.3 shows that modification does produce statistically significant changes in some R wave (leads I, II, III, aVL and aVF) and T wave amplitudes (leads I, II, III, aVL and aVF) at rest. In lead I, electrode modification caused a significant decrease in the amplitudes of the R wave by 0.13 ± 0.01mV (decrease of 21%, $P < 0.001$) and T wave by 0.06mV ± 0.01mV (decrease of 19%, $P < 0.001$) (figure 3.3).

The largest changes in R and T wave amplitudes were in lead III, with increases of 0.27 ± 0.03mV (>33%, $P < 0.001$) in the R wave and 0.12 ± 0.01mV (>74%, $P < 0.001$) in the T wave (figure 3.3). Electrode modification did not cause any T wave amplitudes to exceed their corresponding clinical thresholds for normality (table 2.3, page 60).

R and T wave changes in the frontal plane due to electrode modification were no different during mechanical hyperventilation in normocapnia. Figure 3.4 shows that during mechanical hyperventilation, electrode modification causes similar increases in R wave amplitude of up to 0.26 ± 0.03mV (in lead III; increase of 36%, $P < 0.01$) and T wave amplitude of up to 0.12mV ± 0.01mV (in lead III; increase of 146%, $P < 0.001$) as those seen at rest (figure 3.3). In lead I, electrode modification during mechanical hyperventilation caused a significant

decreases in the amplitude of the R wave by 0.09 ± 0.01mV (decrease of 19%, $P < 0.05$) and T wave by 0.04mV ± 0.01mV (decrease of 17%, $P < 0.01$) (figure 3.4).

Figure 3.1. As expected, modification does not change waveform amplitudes in the transverse plane leads

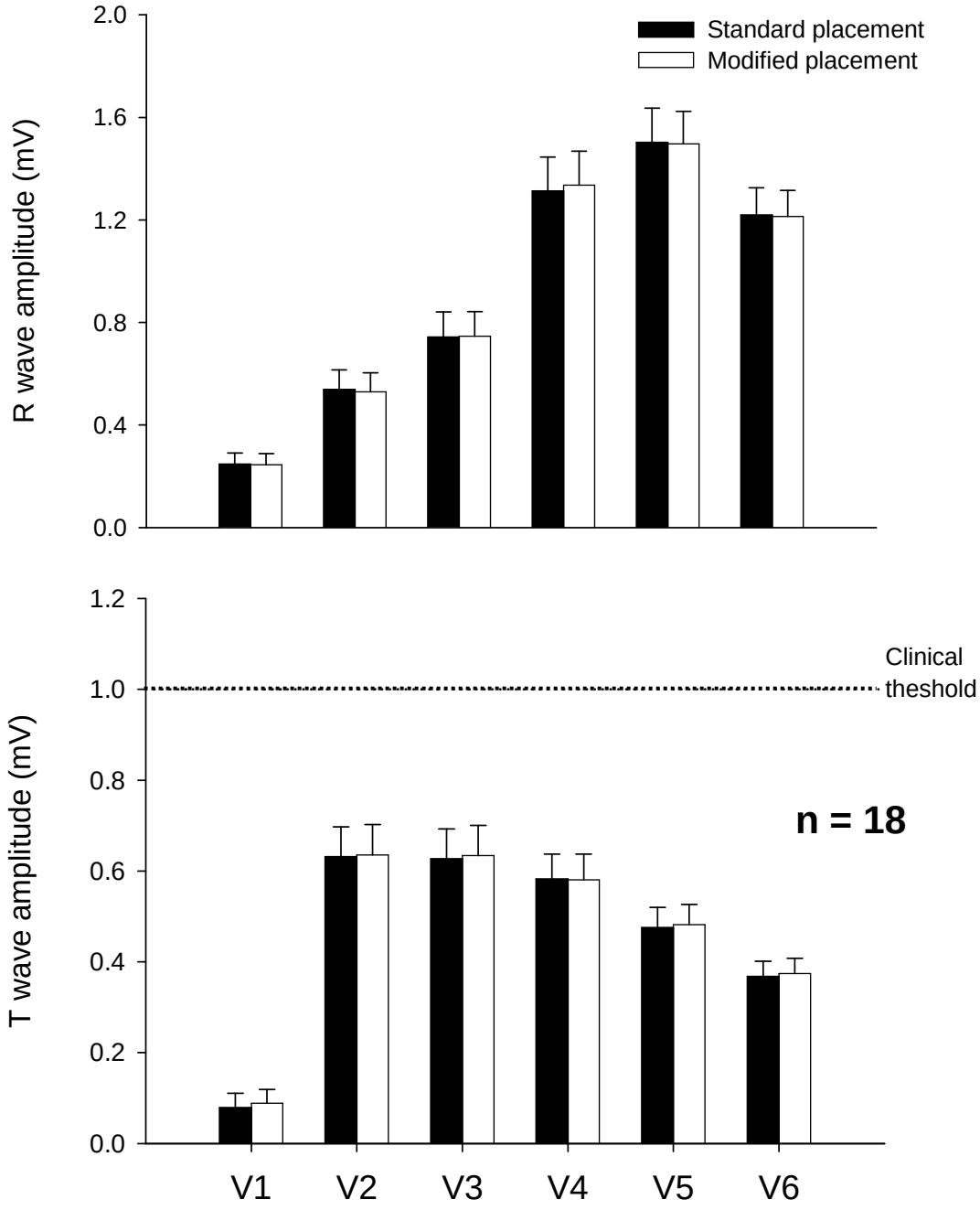


Figure 3.1. Mean ± SE of R and T wave amplitudes in the transverse plane in standard and modified lead placements. Thresholds for clinical abnormality (.....) of T waves (table 2.3) are also shown. All modified placement amplitudes, *NS* vs. standard placement.

Figure 3.2. As expected, modification does not change waveform amplitudes in the transverse plane leads during mechanical hyperventilation in normocapnia

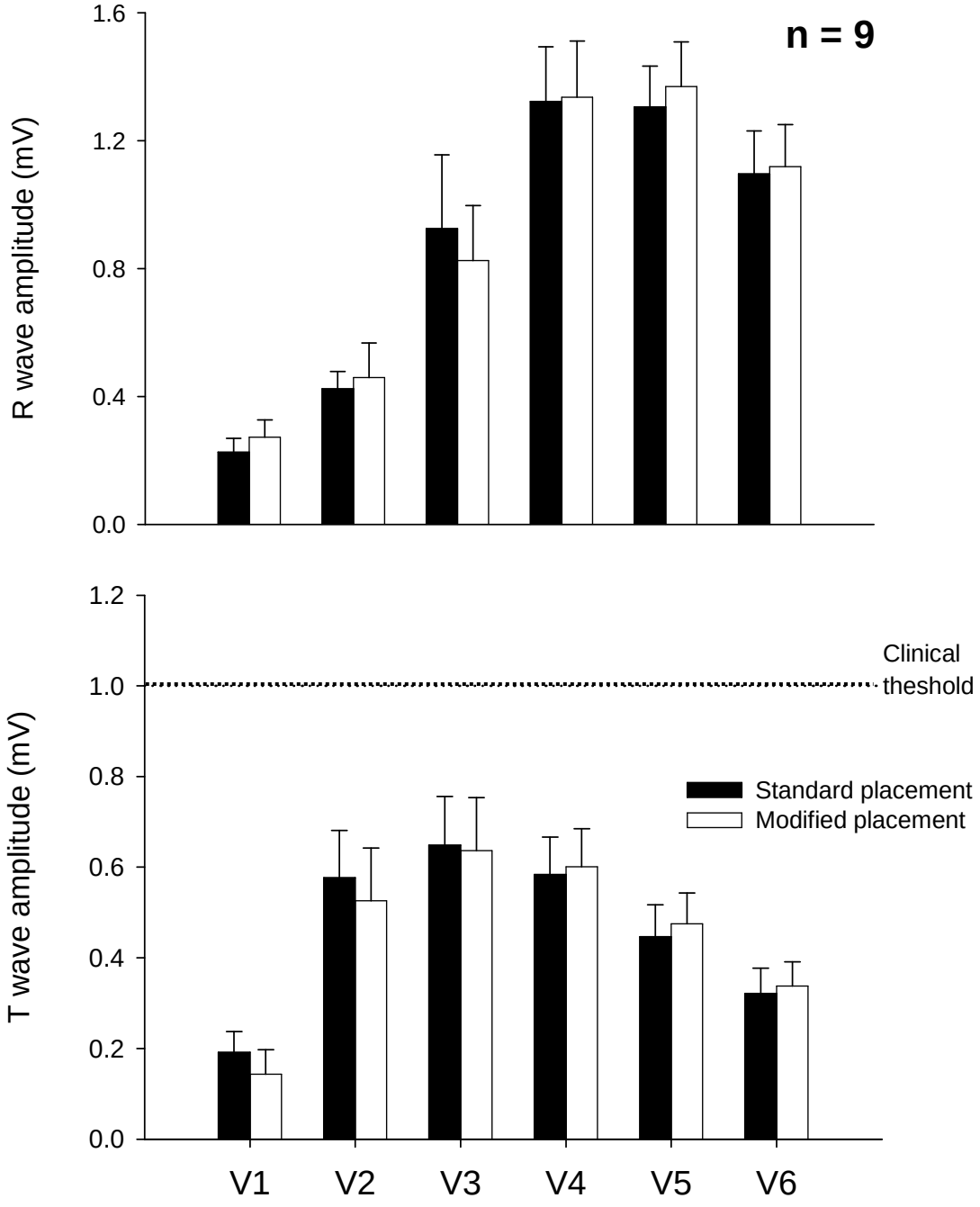


Figure 3.2. Mean ± SE of R and T wave amplitudes in the transverse plane in standard and modified lead placements during mechanical hyperventilation. Thresholds for clinical abnormality (.....) of T waves (table 2.3) are also shown. All modified placement amplitudes, *NS* vs. standard placement.

Figure 3.3. As expected, modification does change waveform amplitudes in the frontal plane leads

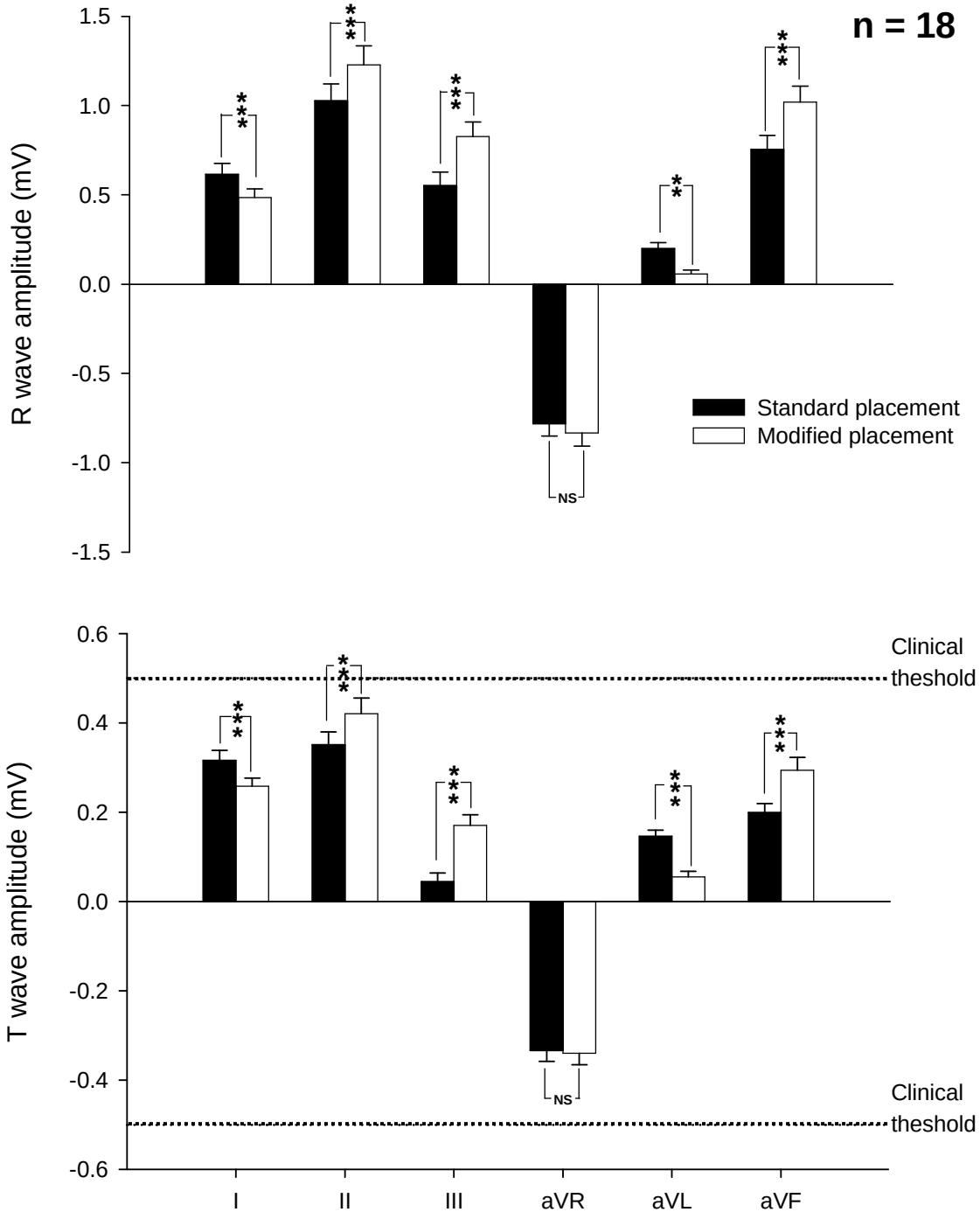


Figure 3.3. Mean ± SE of R and T wave amplitudes in the frontal plane in standard and modified lead placements. Thresholds for clinical abnormality (.....) of T waves (table 2.3) are also shown. * *P* < 0.05, ** *P* < 0.01,* *P* < 0.001, *NS* vs. standard placement.**

Figure 3.4. During mechanical hyperventilation in normocapnia, electrode modification causes similar R and T wave changes in the frontal plane to those seen at rest

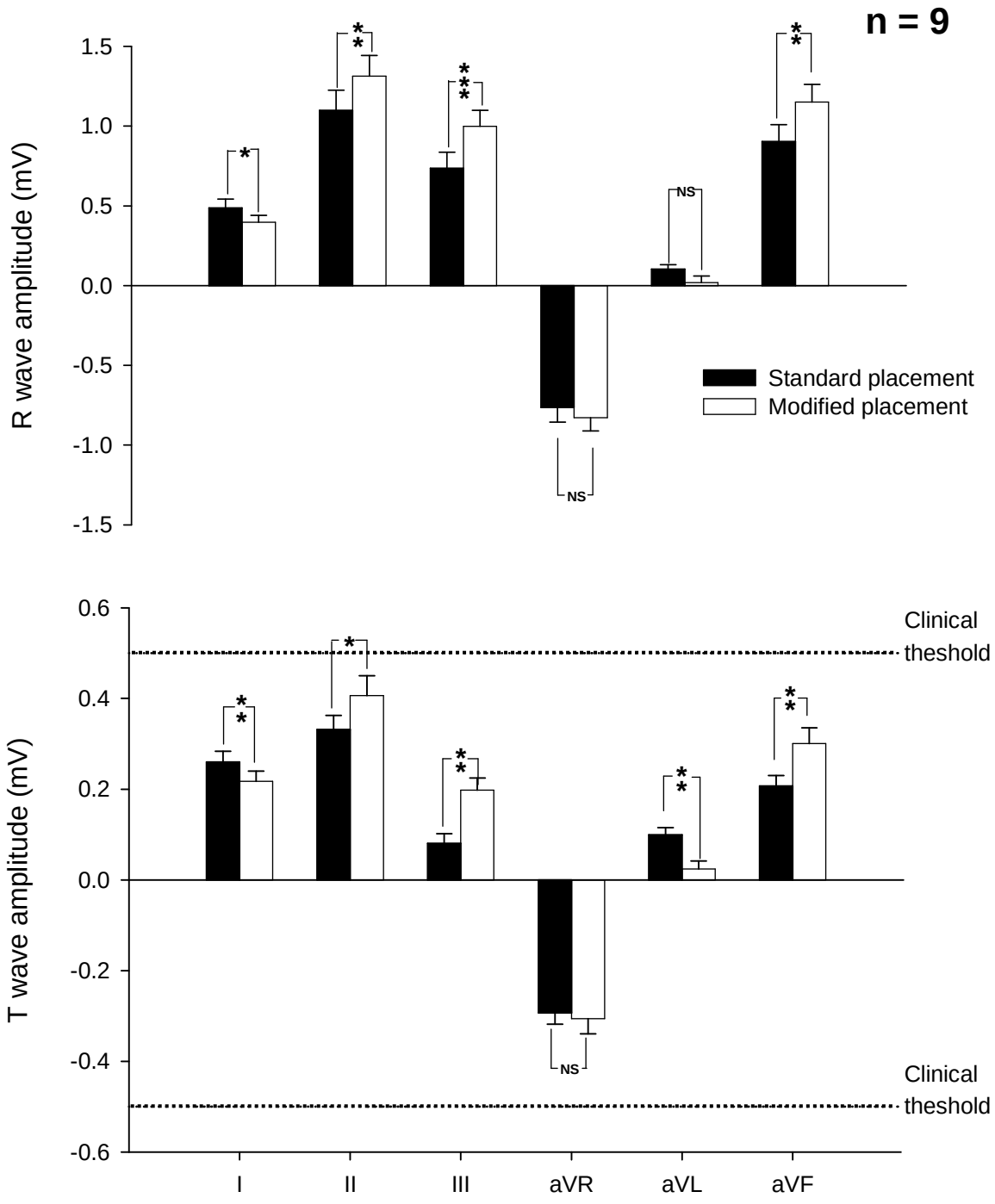


Figure 3.4. Mean ± SE of R and T wave amplitudes in the frontal plane in standard and modified lead placements during mechanical hyperventilation. Thresholds for clinical abnormality (.....) ofT waves (table 2.3) are also shown. * *P* < 0.05, ** *P* < 0.01,* *P* < 0.001, *NS* vs. standard placement.**

Figure 3.5 shows that modification does not produce any statistically or clinically significant changes in ST segment height in either frontal or transverse planes. The largest non-significant increase (0.03 mV) in ST segment height was in lead V_2 ($P > 0.35$) and this was well within the clinical limit ($\geq$ 0.2mV in V_{2-3}) for normality (Wagner *et al.*, 2009). Figure 3.5 shows that ST segment height in lead V_3 was below the clinical threshold for ST depression when electrodes were placed in the standard positions (-0.07 ± 0.04mV). This depressed ST segment was not accompanied by ST(80) point depression or ST flattening.

Figure 3.6 shows modification caused a rightward shift of 14 ± 1° in the QRS axis that is statistically ($P < 0.001$) but not clinically significant (*i.e.* <> -30 to +90°, Surawicz *et al.*, 2009). As expected, modification did not alter the QTc interval duration measured in lead I of the ECG (0.42 ± 0.01 seconds vs. 0.42 ± 0.00 seconds).

Figure 3.7 shows that T wave amplitude (in the transverse plane leads) in the modified electrode placement does not exceed gender specific clinical thresholds for normality in males or females. Figure 3.8 shows that ST segment height (in the transverse plane leads) in the modified electrode placement does not exceed gender specific clinical thresholds for normality in men and women. Once again, ST segment height in lead V_3 (-0.12 ± 0.04mV) (in male subjects) in the standard placement was below the clinical threshold for ST depression (-0.05mV, Wagner *et al.*, 2009) (figure 3.8a). The ST segment in the modified electrode placement was also depressed below clinical thresholds (-0.09 ± 0.03mV) (figure 3.8a). ST segment depression in both leads was not accompanied by ST(80) point depression or ST flattening.

Figure 3.5. Electrode modification did not cause significant ST segment changes in any of the 12 leads of the ECG

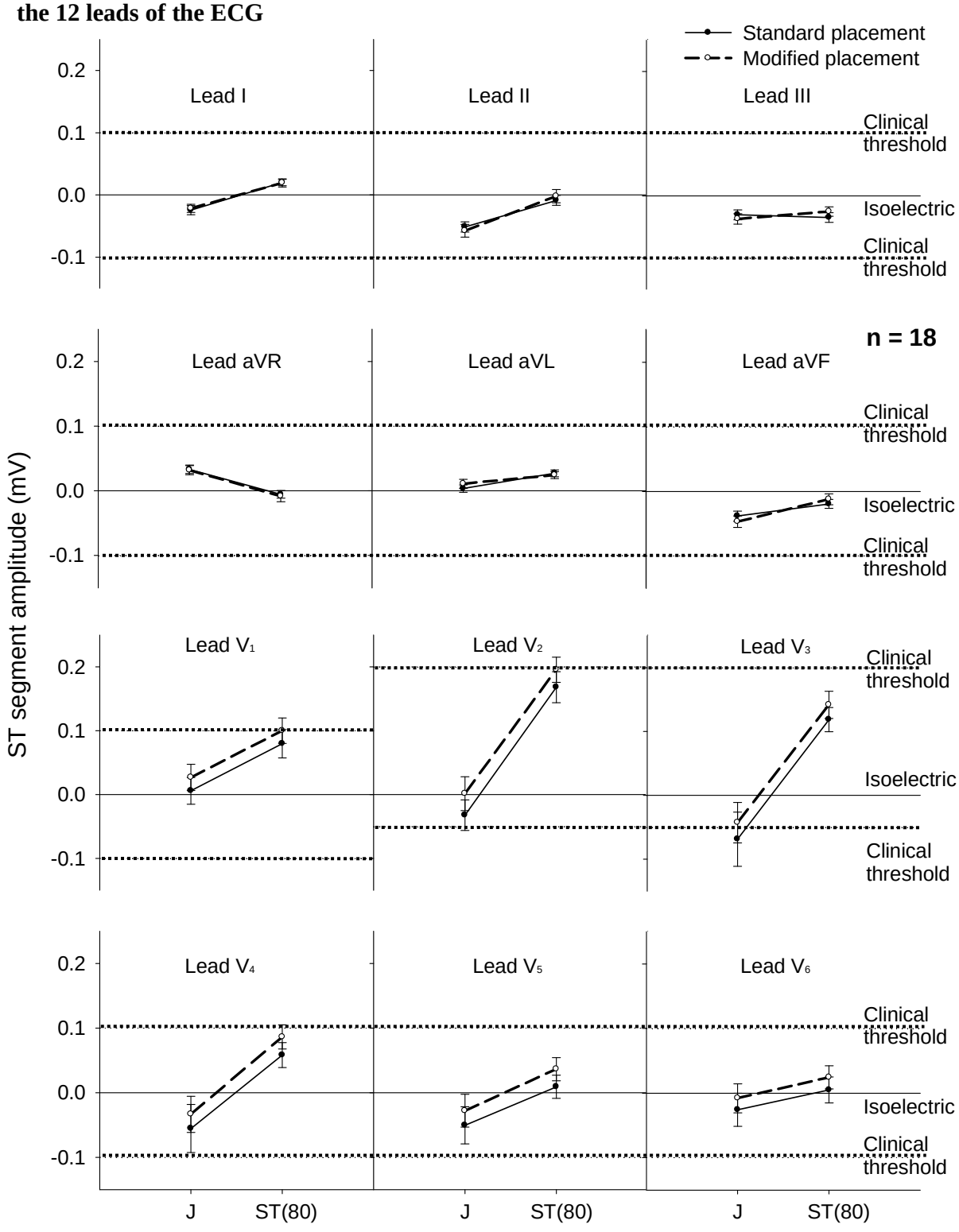


Figure 3.5. ST segments are defined by deviation of the J point from the isoelectric line. The extent of these changes is estimated by deviation of the ST(80) point. J and ST(80) points are presented as means ± standard error. Dotted line represents clinically significant deviation from isoelectric line (straight line). All modified placement ST heights, *NS* vs. standard placement.

Figure 3.6. Modification causes a statistically but not clinically significant rightward shift in the QRS axis

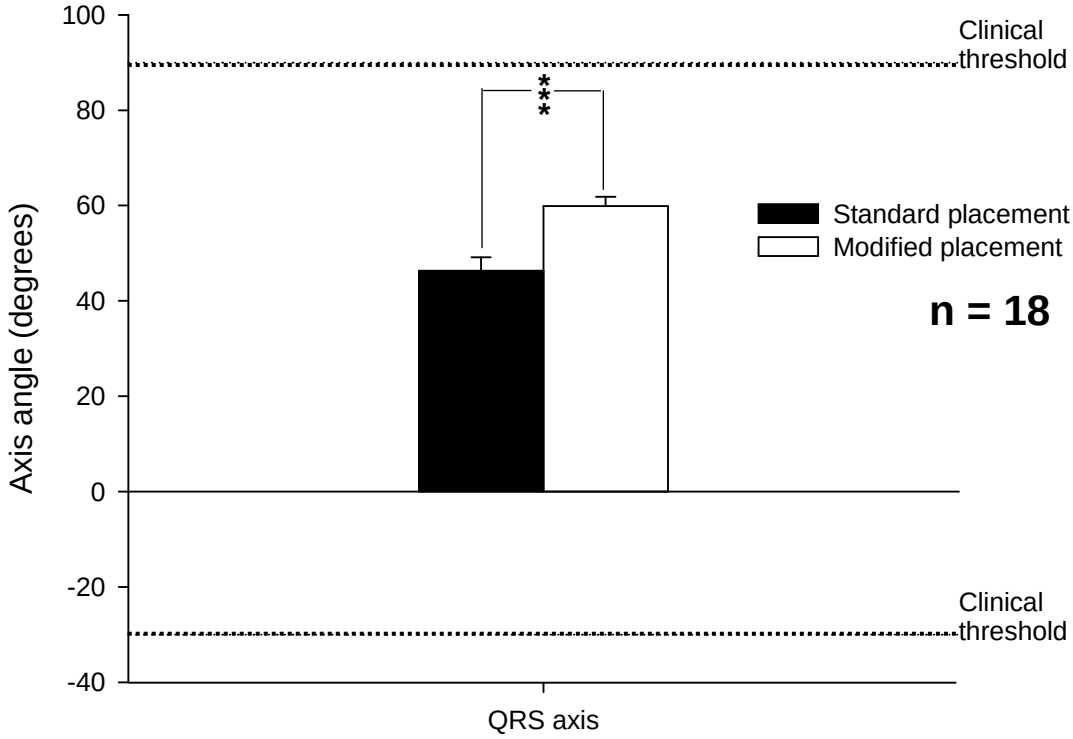


Figure 3.6. Mean + SE of the electrical axes of the QRS complex in both the standard and modified lead placements. Thresholds for clinical abnormality (.....) (table 2.3) shown. Modified placement, * *P* < 0.001 vs. standard placement.**

Figure 3.7. There are no effects of electrode modification in the transverse plane leads when T wave amplitudes are seperated by gender

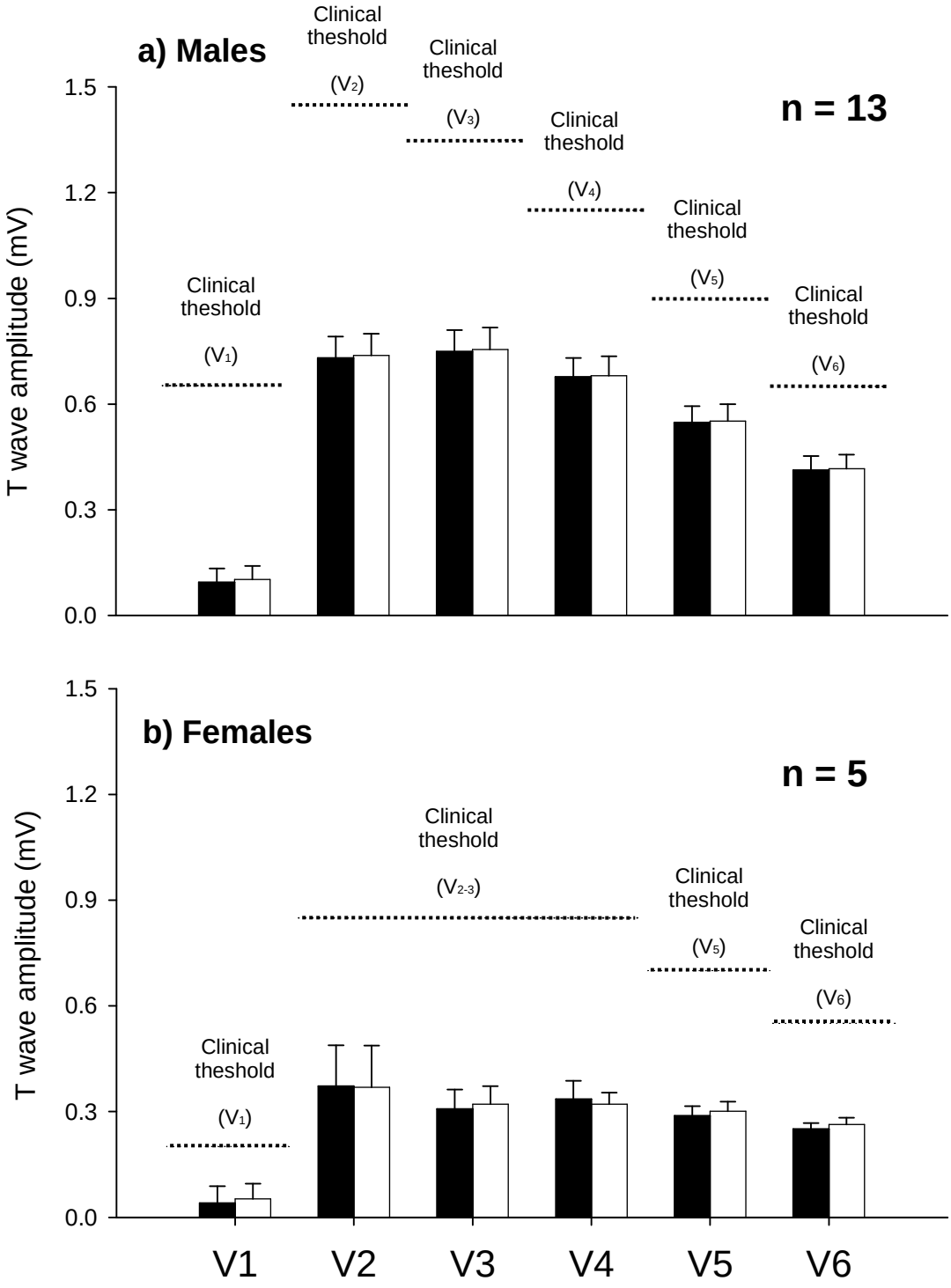


Figure 3.7. Mean ± SE of T wave amplitudes in the transverse plane in standard and modified lead placements in males (a) and females (b). Gender specific clinical thresholds for abnormality (.....) in the T wave (table 2.4) are also shown. All modified placement amplitudes, *NS* vs. standard placement.

Figure 3.8. There are no significant effects of electrode modification in the transverse plane leads when ST heights are seperated by gender

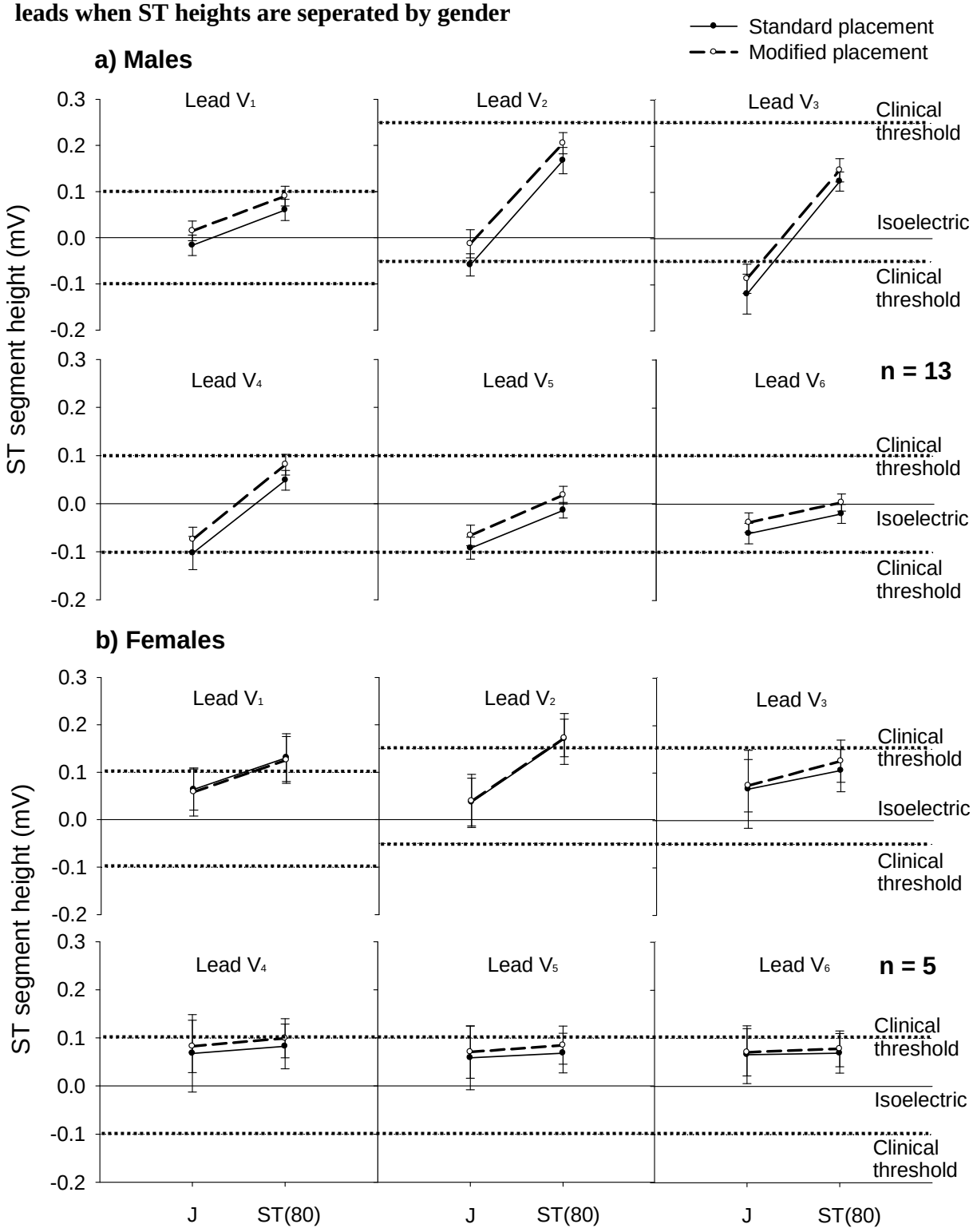


Figure 3.8. Mean ± standard error of ST segment height (J point & ST[80] point) in the transverse plane in males (a) and females (b). Dotted lines are gender sepecific and represent clinically significant deviation from isoelectric line (straight line). All modified placement ST heights, *NS* vs. standard placement.

3.5. Discussion

The modified electrode placement causes statistically significant decreases in both the R wave and T wave of lead I at rest. Statistically significant changes in ECG wave amplitude were only seen in the frontal plane, but electrode modification did not cause these wave amplitudes to exceed clinical thresholds for healthy and semi-recumbent subjects. These changes could also be seen during mechanical hyperventilation in normocapnia. Electrode modification did not cause any changes in the ST segment. Therefore, the modified electrode placement does not reduce the clinical specificity of the 12 lead ECG for detecting myocardial ischemia and may be of use in situations where monitoring the ST segment is of great importance.

Data in the present study were averaged over multiple heart beats, on four separate occasions, in 18 subjects. This maximises the possibility of detecting whether modification causes any small waveform changes that are consistent in one direction. Beat-to-beat changes in ECG waveforms do occur in healthy subjects, for instance when the heart beat happens to coincide with maximum lung inflation (Rutherford *et al.*, 2005). It is therefore important that a large number of heart beats are averaged to produce a balanced representation of the normal waveform. Having shown that modification produces no clinically significant changes, detection of any consistent change in an individual waveform in any new subject would be sufficiently unusual to require further investigation.

3.5.1. Electrode modification in the transverse plane

ECG electrode modification did not cause any statistically significant changes in any of the wave amplitudes measured in the transverse plane at rest or during mechanical hyperventilation (figure 3.1, 3.2, 3.5). As a result, modification also did not cause clinically

important changes in these leads. Separating data by gender did not reveal any hidden variation between standard and modified electrode placements in the transverse plane. This lack of changes was expected because electrode modification does not involve altering any of the precordial electrode positions. These findings are contrary to some previous studies that have reported changes in the transverse plane leads of up to 0.04mV in the R wave (due to alteration in the 'position' of the central terminal reference point) (Edenbrandt *et al.*, 1989; Mason & Likar, 1966; Rautaharju *et al.*, 1980; Krucoff *et al.*, 1994). These changes however, were always within 50% of standard values (table 3.3).

As expected, electrode modification did not cause any significant changes in the QTc interval duration. This confirms the suggestion by Kligfield *et al.,* (2007) that electrode modification does not adversely affect rhythm diagnosis in the ECG.

3.5.2. Electrode modification in the frontal plane

In the frontal plane leads, one would expect modification to alter the ECG waveform because the limb electrodes are moved onto the torso. Statistically significant changes in R and T wave amplitudes were observed in all these leads except of in aVR (figure 3.3). Changes in leads I, II and aVF were <50% and this was in accordance with previous studies (Edenbrandt *et al.*, 1989; Gamble *et al.*, 1984; Mason & Likar, 1966; Papouchado *et al.*, 1987; Rautaharju *et al.*, 1980). The findings of previous studies that modification does significantly alter the QRS axis (by 14 $\pm$ 1°) in the frontal plane was also confirmed (figure 3.6) (Edenbrandt *et al.*, 1989; Gamble *et al.*, 1984; Papouchado *et al.*, 1987; Rautaharju *et al.*, 1980; Jowett *et al.*, 2005; Kleiner *et al.*, 1978; Sevilla *et al.*, 1989; Takuma *et al.*, 1995).

The modified electrode placement causes statistically significant decreases of ~20% in both the R and T wave of lead I at rest. This effect was also evident during mechanical hyperventilation in normocapnia. The effects of electrode modification on the amplitudes of the R and T waves in lead I were of interest in this thesis investigation because Rutherford *et al.,* (2005) used the modified electrode placement to study the effects of hypocapnia, induced by mechanical hyperventilation, on the ECG. The findings of the present study suggest that the modified electrode placement used by Rutherford *et al.,* (2005) may have significantly altered the absolute R and T wave amplitudes in their study. Subsequent studies in this thesis investigation will therefore use the standard electrode placement in order the measure true wave amplitude changes during mechanical hyperventilation in normocapnia and hypocapnia.

3.5.3. Electrode modification and the ST segment

The ST segment is used as a key indicator of myocardial ischemia/infarction during exercise stress testing and emergency monitoring. This study shows that modification does not change the ST segment in any leads (figure 3.5, 3.8). ST depression beyond the clinical threshold for normality was observed in lead V_3 when leads were placed in the standard electrode placement. This was not thought to be of interest because ST segment depression was not accompanied by ST(80) depression or ST flattening, recordings were made in the standard positions in clinically normal subjects at rest and ST segment depression did not occur in any other leads (for ST segment depression to be of clinical importance it must occur in two anatomically contiguous leads, Wagner *et al.*, 2009).

The largest non-statistically significant increase in ST height was only 0.03 mV (V_2), which is similar to that found with the modification of Krucoff *et al.,* (1994). Although Weins &

Chaitman (1997) and Jowett *et al.*, (2005) claimed that the Takuma modification (used in this study) did cause ST elevation in both the inferior and lateral leads to such a degree that false diagnosis of myocardial injury could occur, no numerical data was provided. Other modifications may also produce clinically important ST segment changes (Rautaharju *et al.*, 1980; Gamble *et al.*, 1984), although it appears their ST changes may be within the most up to date clinical boundaries (Wagner *et al.*, 2009). This study therefore provides unequivocal evidence that electrode modification does not cause clinically significant changes in the ST segment, and thus does not introduce false positive ischemic ECG changes.

3.5.4. Conclusions

No previous study has quantified the precise differences in amplitude in every waveform and found an objective means of judging whether such differences matter. This study addresses this issue using an ECG apparatus that improves confidence in wave amplitude measurements and compares any differences with the latest AHA, ACCF and HRS guidelines on the clinical thresholds for abnormality in the ECG (table 2.3; Wagner, 2008; Wagner *et al.*, 2009; Rautaharju *et al.*, 2009; Surawicz *et al.*, 2009). The study shows that wave amplitude changes caused by electrode modification are sufficiently small to remain within the clinical limits for normality. These small changes may be of importance in a research setting such as when studying the subtle effects of hypocapnia in normal subjects. However, because all ECG wave amplitude changes were within the clinical limits for normality, it can be concluded that modification does not affect the clinical specificity for myocardial ischemia of the 12 lead ECG.

This investigation does not attempt to test whether electrode modification alters the sensitivity of the standard ECG in patients with known ECG abnormalities. If modification produced similar changes in these patients, it could alter sensitivity. For instance, diagnosis of a new onset bundle branch block relies, in part, on assessment of the QRS axis. In an acute myocardial infarction situation with a borderline axis, the change caused by modification in the present study would be of critical importance for immediate patient management.

Chapter 4

Does hypocapnia, induced by mechanical hyperventilation, cause a significant change in cardiac electrical activity or heart function in normal healthy subjects?

4.1. Summary

The effects of the most severe hypocapnia tolerable (20 ± 0mmHg), induced by mechanical hyperventilation, on cardiac electrical activity and heart function were examined in 18 normal healthy resting subjects. Cardiac electrical activity was assessed by a standard 12 lead ECG and heart function was measured using 3 modes of echocardiography (M-mode, Doppler blood flow and tissue Doppler echocardiography).

Hypocapnia only caused a significant increase in T wave amplitude in the anteroseptal leads (V_{1-3}) of the ECG (up to 0.9mV [15%]). This elevation did not cause any T wave amplitude to increase beyond the clinical limits for normality set out by the AHA, ACCF and HRS (Rautaharju *et al.*, 2009; Wagner, 2008). Hypocapnia had no effect on R wave amplitude, ST segment height or QTc interval duration in any leads.

Hypocapnia did not cause any significant changes in LVID, FS, E/A ratio, deceleration time or diastolic septal and lateral wall velocities measured by echocardiography.

The findings of this study suggest that mechanically induced hypocapnia (20 ± 0mmHg) does not induce ischemic changes in the heart (measured by electrocardiography and echocardiography) in normal healthy subjects. These findings do not discount the possibility

of ischemic ECG changes occurring in patients suffering from coronary artery disease. However, they do suggest that the overall effect is not as severe as was previously thought.

4.2. Introduction

Hypocapnia causes coronary vasoconstriction and an increased affinity of haemoglobin for O_2, both of which decrease O_2 delivery to the myocardium (Laffey & Kavanagh, 2002; Neill & Hattenhauer, 1975; Rutherford *et al.*, 2005). Animal studies have shown that hypocapnia (23mmHg), induced by mechanical hyperventilation, causes large reductions in myocardial blood flow to the septum (by 44%), right ventricle wall (by 50%) and left ventricle wall (by 44%) in anaesthetized pigs (Karlsson *et al.*, 1994) and reductions in coronary flow of 23-33% in anaesthetized dogs (Coetzee *et al.*, 1984; Vance *et al.*, 1973). In normal healthy humans, hypocapnia (19-20mmHg) induced by voluntary hyperventilation, has been shown to reduce coronary blood flow by 30% which is thought to be caused by coronary vasoconstriction (Rowe *et al.*, 1962; Yokoyama *et al.*, 2008).

Hypocapnia causes an increased affinity of haemoglobin for oxygen (Roughton, 1964). This shift of the oxygen dissociation curve to the left is known as the Bohr shift (figure 1.1, page 13). This is caused by the direct effect of decreased CO_2 on haemoglobin and an increase in arterial blood pH caused by decreased $PaCO_2$ (Roughton, 1964). In humans, this effect has been demonstrated by a higher than expected partial pressure of O_2 in the coronary sinus (venous) blood during hypocapnia (19mmHg) (Neill & Hattenhauer, 1975).

This investigation aims to test whether hypocapnia can cause myocardial ischemia, which in turn, can induce noticeable, clinically significant ECG changes in normal healthy subjects. If

this is the case, hypocapnia induced by mechanical hyperventilation may be of clinical value for use in the early diagnosis of coronary artery disease (*i.e.* hypocapnia could be used as a provocation test in patients with suspected coronary artery disease). The aim of a provocation test is to stimulate noticeable changes in a patient population which do not occur in normal healthy subjects. The advantage of a provocation test that produces additional small and consistent changes in a normal population is that it validates any negative results (*i.e.* the provocation worked but did not cause large changes because the disease was not present). It was therefore of interest to see whether hypocapnia can produce small but repeatable ischemic changes in normal subjects that become accentuated further in patient populations.

4.2.1. Voluntary hyperventilation and the ECG

In normal subjects, hypocapnia (14-24mmHg), induced by voluntary hyperventilation, has been shown to cause inconsistent changes in the T wave, ST segment and QTc interval in 8-57% of subjects (table 1.2, page 19) (Barker *et al.*, 1939; Biberman *et al.*, 1971; Christensen, 1946; Golden *et al.*, 1975; Joy & Trump, 1981; Kemp & Ellestad, 1968; Lary & Goldschlager, 1974; McCance, 1932; Scherf & Schlachman, 1947; Thompson, 1943; Wasserberger *et al.*, 1956). Because voluntary hyperventilation does not allow breathing frequency or tidal volume to be accurately controlled, it is difficult to induce a consistent level of hypocapnia that is severe enough to induce changes in O_2 delivery to the heart. Few studies have quantified the level of hypocapnia induced and in those that do, it differs by as much as 10mmHg (Biberman *et al.*, 1971; Golden *et al.*, 1975). Inconsistent findings may be caused by differing levels of hypocapnia induced by voluntary hyperventilation.

Inconsistent inflation volumes also affect ECG changes during hyperventilation. Rutherford *et al.*, (2005) demonstrated that increasing inflation volume by 0.6 litres increases the distance between the heart and ECG electrodes causing the size of the T wave and R wave in lead I to decrease by 0.2-0.3mV. The effects of varying inflation volumes on ECG wave amplitudes can been seen in studies where similar R wave decreases are reported with T wave depression (Barker *et al.*, 1939; Christensen, 1946; Scherf & Schlachman, 1947).

Voluntary hyperventilation increases physical exertion and metabolic rate due to increased work of the respiratory muscles. This means more CO_2 is produced than at rest, making hypocapnia more difficult to induce. This increased physical exertion also limits the duration one can comfortably hyperventilate for. Previously, subjects have voluntarily hyperventilated for between 10 seconds and 16 minutes (Barker *et al.*, 1939; Biberman *et al.*, 1971; Christensen, 1946; Golden *et al.*, 1975; Joy & Trump, 1981; Kemp & Ellestad, 1968; Lary & Goldschlager, 1974; Scherf & Schlachman, 1947; Thompson, 1943; Wasserberger *et al.*, 1956). It is not clear how long (or to what degree) hypocapnia was maintained for during these periods. It is unlikely that hyperventilating for 10 seconds would be long enough to induce a significant level of hypocapnia, and therefore, the true effects of hypocapnia may not have been revealed.

Voluntary hyperventilation may also cause changes in plasma electrolyte concentration. The extent of these changes is unclear and data from previous studies are confusing. It is thought that voluntary hyperventilation causes increases (of 0.3-0.5mEq/L from 3.6-4.6mEq/L) (Yu *et al.*, 1959; Biberman *et al.*, 1971; Krapf *et al.*, 1995) or decreases in blood potassium (of 0.2mEq/L from 4mEq/L) (Mostellar & Tuttle, 1964) and decreases in blood calcium (of

0.1mg/100ml from 10-10.5 ± 0.1mg/100ml) (Yu *et al.*, 1959). However, the electrolyte changes in these studies appear to be within the normal range for both K^+ (4-5mEq/L) and Ca^{2+} (9-11mg/100ml) (Wagner, 2008). It is possible that changes in plasma electrolytes are caused by sympathetic activation which also causes changes in heart rate (increase of 9-58bpm) (Biberman *et al.*, 1971; Christensen, 1946; Joy & Trump, 1981; Mcgregor *et al.*, 1962; Miyagi *et al.*, 1989; Previtali *et al.*, 1989; Richardson *et al.*, 1972; Burnum *et al.*, 1954). Changes in plasma electrolytes would be of interest because hypokalemia causes ECG changes such as T wave inversion (-0.15mV), ST depression (-0.1mV) and QTc interval prolongation (>0.43 seconds) that are not of ischemic origin (Wagner, 2008). Hyperkalemia causes hyperacute T waves (>0.5-1.0mV) and hypocalcemia causes QTc interval prolongation. Rutherford *et al.*, (2005) showed the hypocapnia induced by mechanical hyperventilation does not cause significant changes in plasma electrolytes. Here, venous blood samples were taken during mechanical hyperventilation in normocapnia and hypocapnia to confirm this. Heart rate and mean arterial pressure were also recorded continuously, to confirm that subjects were relaxed throughout mechanical hyperventilation and that hypocapnia causes only trivial (if any) changes in heart rate and blood pressure.

Because of the difficultly in separating the effects of hypocapnia on the ECG from the effects of changing inflation volumes, inconsistent breathing frequency and physical exertion, voluntary hyperventilation is not considered a reliable method for studying the effects of hypocapnia on cardiac electrical activity (Rutherford *et al.*, 2005).

4.2.2. Mechanical hyperventilation and the ECG

Mechanical hyperventilation via face mask produces consistent and reproducible changes in $PetCO_2$ that can be accurately controlled (Cooper *et al.*, 2004). It allows hypocapnia to be induced at a constant tidal volume and set breathing frequency, eliminating the effects of varying inflation volumes on the wave amplitudes of the ECG. Mechanical hyperventilation requires no physical exertion, allowing sustained periods of hypocapnia to be induced without causing discomfort to the subject.

A reduction in T wave amplitude, thought to be caused by decreased O_2 availability in the myocardium, has been demonstrated previously in normal healthy subjects during hypocapnia induced by mechanical hyperventilation (Rutherford *et al.*, 2005). In 13/15 subjects (87%), hypocapnia (20mmHg) was shown to cause a significant reduction in T wave amplitude of 0.1 ± 0.0mV in lead I, independently of R wave changes. They also showed that hypocapnia and mechanical hyperventilation have no effect on plasma K^+ or Ca^{2+} concentrations. This was the first study to provide irrefutable evidence that hypocapnia causes a reduction in T wave amplitude, even in normal healthy subjects. This experiment was performed using a 3 lead ECG (ECG waveform recorded in lead I) with ECG electrodes positioned in the modified electrode placement. As yet, these findings have not been replicated nor reproduced using a 12 lead ECG. Measuring the effects of hypocapnia on a 12 lead ECG would be of interest because it permits a more comprehensive assessment of the global affects of hypocapnia on cardiac activity.

This investigation aimed to establish whether T wave changes seen during hypocapnia induced by mechanical hyperventilation in lead I of the ECG can be reproduced in a 12 lead

ECG. In addition to measures of R and T wave amplitude, supplementary indicators of myocardial ischemia such as ST segment height and QTc interval duration were also measured. All waveform amplitudes and interval durations were compared to AHA, ACCF and HRS clinical guidelines for myocardial ischemia (table 2.3, page 60) (Rautaharju *et al.*, 2009; Kenigsberg *et al.*, 2007; Wagner, 2008; 2009). ST segment height and T wave amplitude recorded in the transverse plane were also separated by gender and compared to gender specific clinical thresholds, for reasons previously described (chapters 2.9.1, page 47).

4.2.3. Hypocapnia and the echocardiogram

Echocardiography was used in the present investigation as an independent measure of any potentially ischemic changes in heart function observed by electrocardiography during hypocapnia. Echocardiography is a useful tool for identification of myocardial ischemia in patients suffering from coronary artery disease (Fujii *et al.*, 1988; Garcia-Fernandez *et al.*, 1999; Kostkiewicz *et al.*, 2003; Nishimura & Tajik, 1997).

Echocardiography has rarely been used to investigate the effects of hypocapnia on heart function. Fujii *et al.*, (1988) used echocardiography and voluntary hyperventilation to assess wall motion abnormalities in 27 patients with variant angina. They measured wall motion with two-dimensional m-mode echocardiography recorded from the standard parasternal/subcostal short-axis view of the left ventricle at the level of the papillary muscles. They found measurable decreases in wall motion and systolic wall thickening (no data presented) and these were associated with ST segment deviation.

In the present investigation, two-dimensional directed m-mode echocardiography, Doppler blood flow analysis of global diastolic function and tissue Doppler measures of left ventricular wall velocity were used to identify signs of myocardial ischemia during hypocapnia.

4.3. Methods

Eighteen normal healthy subjects aged 24 ± 3 years old (20-30 years old) (13 male) with no known cardiovascular disease were tested on three separate occasions on different days. Subjects gave informed consent and all experiments were approved by the Walsall Local Research Ethics Committee. All experiments were performed in accordance with the Declaration of Helsinki as stated by the American Physiological Society (2002). During each visit, subjects lay semi-recumbent on a bed and listened to the radio through headphones.

In all experiments, continuous blood pressure was recorded using a finger plethysmograph (Finapres 2300) and end-tidal CO_2 was recorded from expiration through an in-line capnograph (Hewlett Packard 78354A). To confirm the effects of hypocapnia on plasma K^+, Ca^{2+} and pH, venous blood samples were taken from the antecubital vein in 14 subjects during each condition and analysed using a Rapid Lab 865 analyzer.

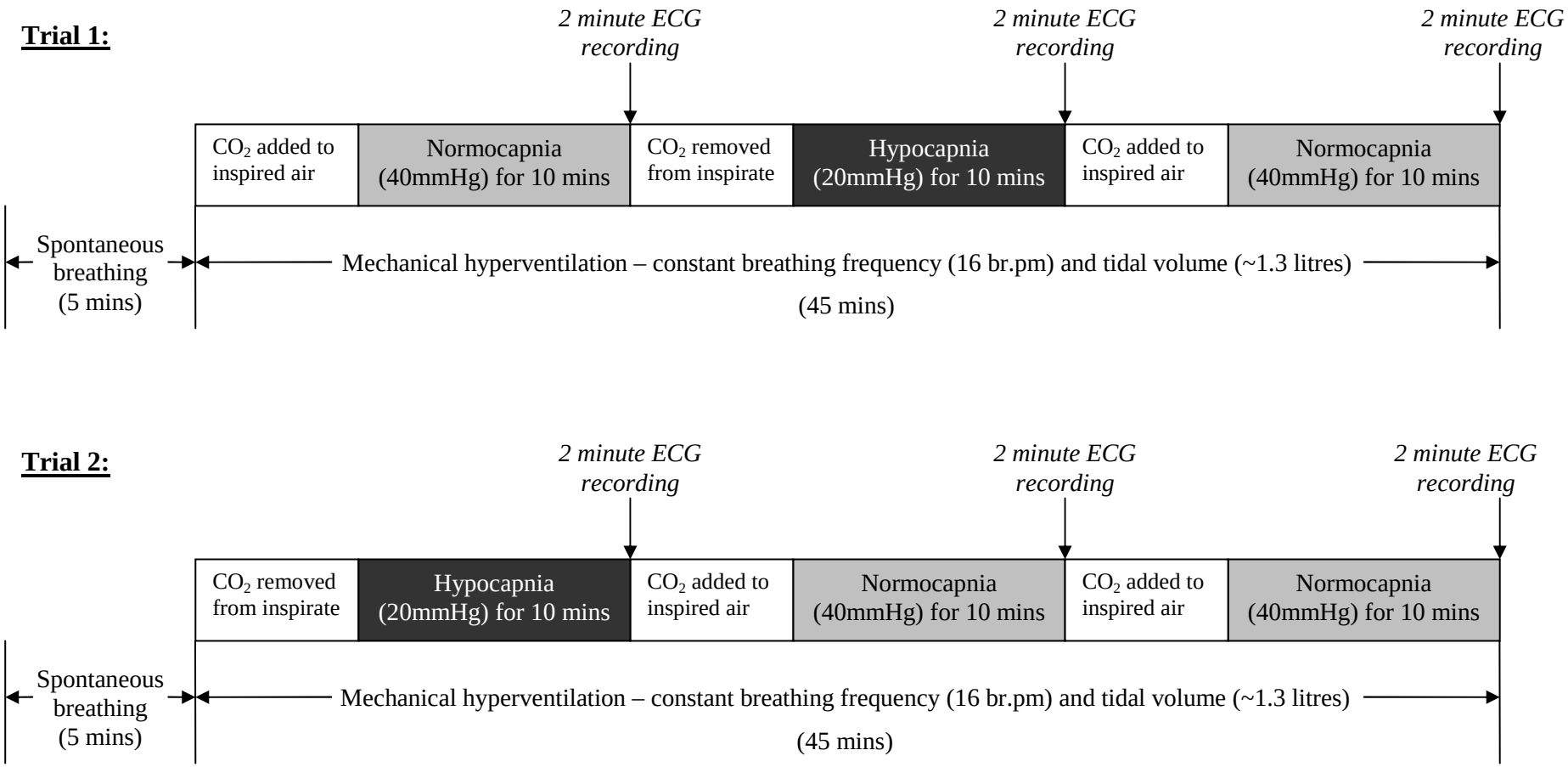
Mechanical hyperventilation was performed at baseline with normal $PetCO_2$ (normocapnia) and reduced $PetCO_2$ (hypocapnia) using a positive pressure mechanical ventilator (Engstrom Erica II) as previously described (chapter 2.3, page 26). Breathing frequency (16 br.pm) and tidal volume (~1.3litres) were kept constant throughout both conditions during mechanical hyperventilation. It is known that hyperventilation decreases the amplitude of the ECG

waveform due to increased inflation volume (Rutherford *et al.*, 2005). It was therefore expected that ECG waveforms would decrease between spontaneous breathing and normocapnia. It was not the aim of the present investigation to examine this phenomenon further; therefore ECG waveforms were not analysed during spontaneous breathing prior to mechanical hyperventilation.

12 lead ECGs were recorded in 18 subjects during normocapnia and hypocapnia in two separate trials, using the ECG apparatus previously described (chapter 2.9.2, page 50). All ECG electrodes were placed in the standard electrode placement defined in Kligfield *et al.*, (2007) (figure 2.4, page 43). The order that normocapnia and hypocapnia were induced was altered between trials to remove any possible effect of order or duration of ventilation on the recorded ECG waveforms (figure 4.1).

An average ECG waveform was calculated from each two minute recording in each lead and each condition (as previously described in chapter 2.9.3, page 55). Further analysis scripts were used to calculate the amplitudes of the R and T waves, ST segment height and QTc interval duration. Two-dimensional m-mode, Doppler blood flow and tissue Doppler echocardiographic measurements were recorded in both normocapnia and hypocapnia in 18 subjects during a third trial. LVID in diastole and systole (in 2D M-mode), fractional shortening (in 2D M-mode), E/A ratio (in Doppler blood flow mode) and myocardial wall velocities (in tissue Doppler mode) were measured as indicators of myocardial ischemia during hypocapnia.

Figure 4.1. Order and duration that normocapnia and hypocapnia were induced during each trial in mechanical hyperventilation



Data from both recordings in normocapnia in each trial were averaged. ECG and echocardiographic data is presented with non-gender specific clinical thresholds for normality (Wagner, 2008; Rautaharju *et al.*, 2009; Surawicz *et al.*, 2009; Wagner *et al.*, 2009; Salcedo, 1978; Weyman, 1982; Hamlin *et al.*, 2004; Garcia *et al.*, 1998; Garcia-Fernandez *et al.*, 1999; Garcia *et al.*, 1998; Kostkiewicz *et al.*, 2003). ST segment height and T wave amplitude (recorded in the transverse plane) were also separated by gender and compared to gender specific clinical thresholds as recommend by Wagner *et al.*, (2009) and Rautaharju *et al.*, (2009).

All data are expressed as means ± standard error for 18 subjects. To identify the statistical significance between wave amplitudes and QTc interval duration recorded in normocapnia and hypocapnia, a 2-tail paired t-test was performed in each lead with application of the Bonferroni correction for multiple (12) comparisons. The Bonferroni correction assumes that all 12 measurements are independent and uncorrelated. The difference between measures of LVID in diastole and systole, fractional shortening, E/A ratio and myocardial wall velocities in each condition were calculated by a 2-tail paired t-test without correction.

4.4. Results

In all subjects, mechanical hyperventilation, without the addition of CO_2 to inspired air, caused $PetCO_2$ to decrease by 18 ± 0mmHg (figure 4.2). This caused an increase in venous blood pH by 0.2 ± 0.0 pH units to 7.6 (figure 4.3). Hypocapnia did not cause significant changes in venous blood K^+ concentration (figure 4.4). Hypocapnia caused small decreases in venous Ca^{2+} concentration (by 0.03 ± 0.00mmol/l, $P < 0.001$) but these were within the normal clinical range for Ca^{2+} changes that affect the ECG (figure 4.4). Hypocapnia caused

decreases in mean arterial pressure (by 9 ± 3mmHg, $P < 0.01$) (figure 4.5) and increases in heart rate (by 4 ± 1 bpm, $P < 0.01$]).

Figure 4.2. $PetCO_2$ decreases by 18 mmHg during mechanical hyperventilation in hypocapnia in all subjects

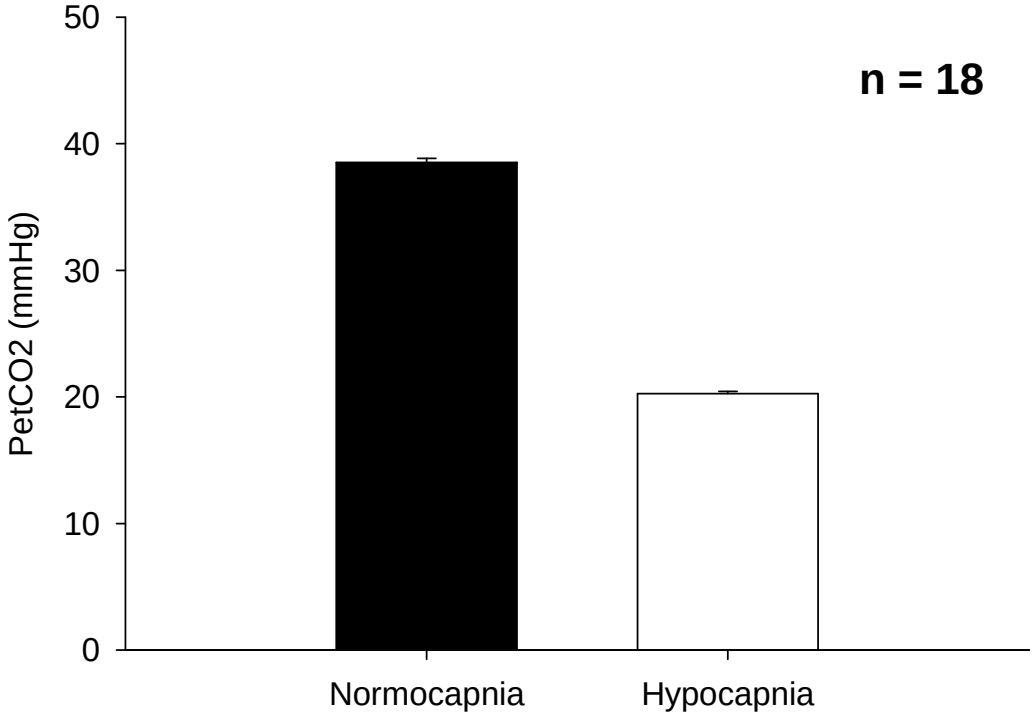


Figure 4.2. Mean ± SE $PetCO_2$ during mechanical hyperventilation in normocapnia and hypocapnia in 18 subjects.

Figure 4.3. Hypocapnia increase venous blood pH in all subjects during mechanical hyperventilation

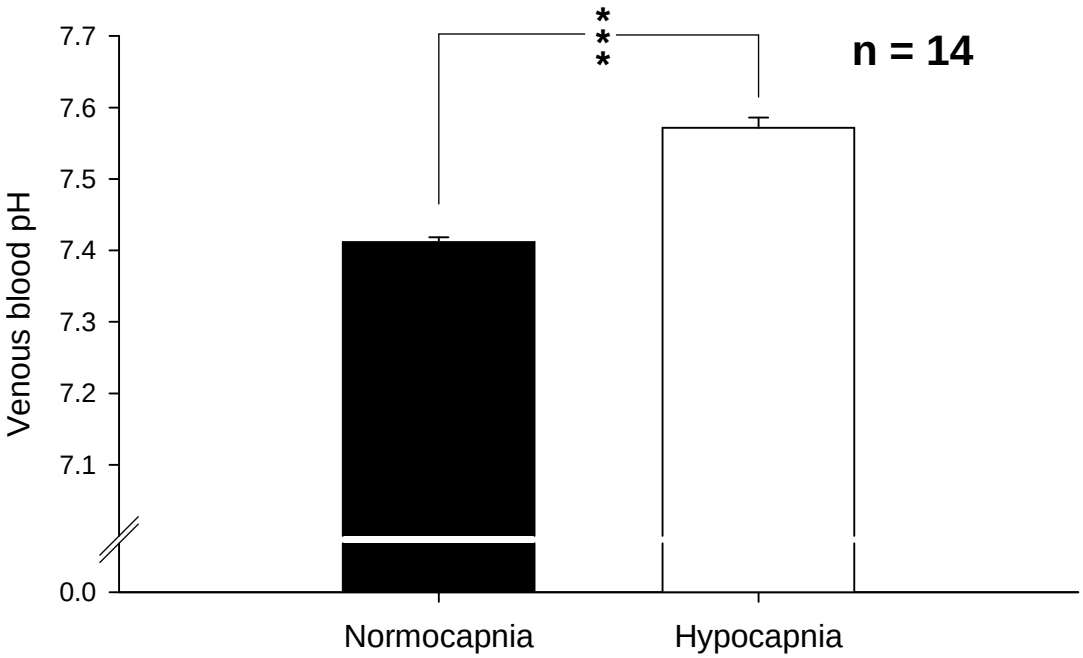


Figure 4.3. Mean ± SE venous blood pH in venous blood during mechanical hyperventilation in normocapnia and hypocapnia.* *P* < 0.001 vs. normocapnia**

Figure 4.4. Hypocapnia causes small changes in venous blood electrolyte concentrations in 14 normal subjects

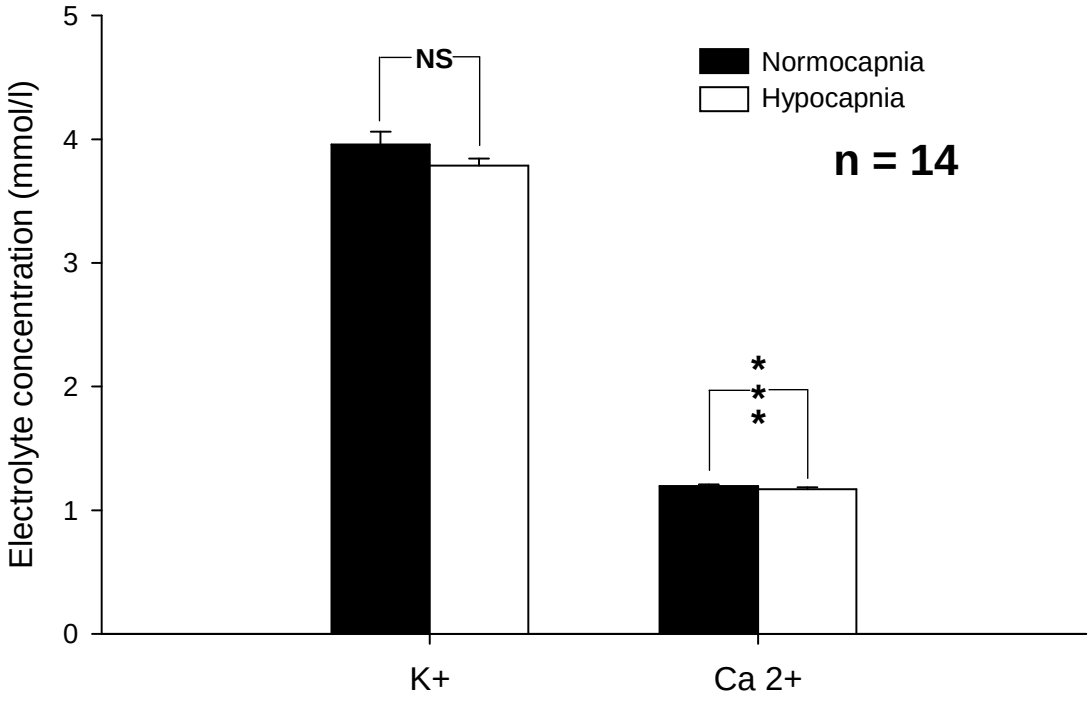


Figure 4.4. Mean ± SE potassium and calcium ion concentrations in venous blood during mechanical hyperventilation in normocapnia and hypocapnia. Values in hypocapnia, * *P* < 0.001 or *NS* vs. normocapnia**

Figure 4.5. Hypocapnia causes a significant increase in heart rate and significant decrease in mean arterial pressure in all subjects during mechanical hyperventilation

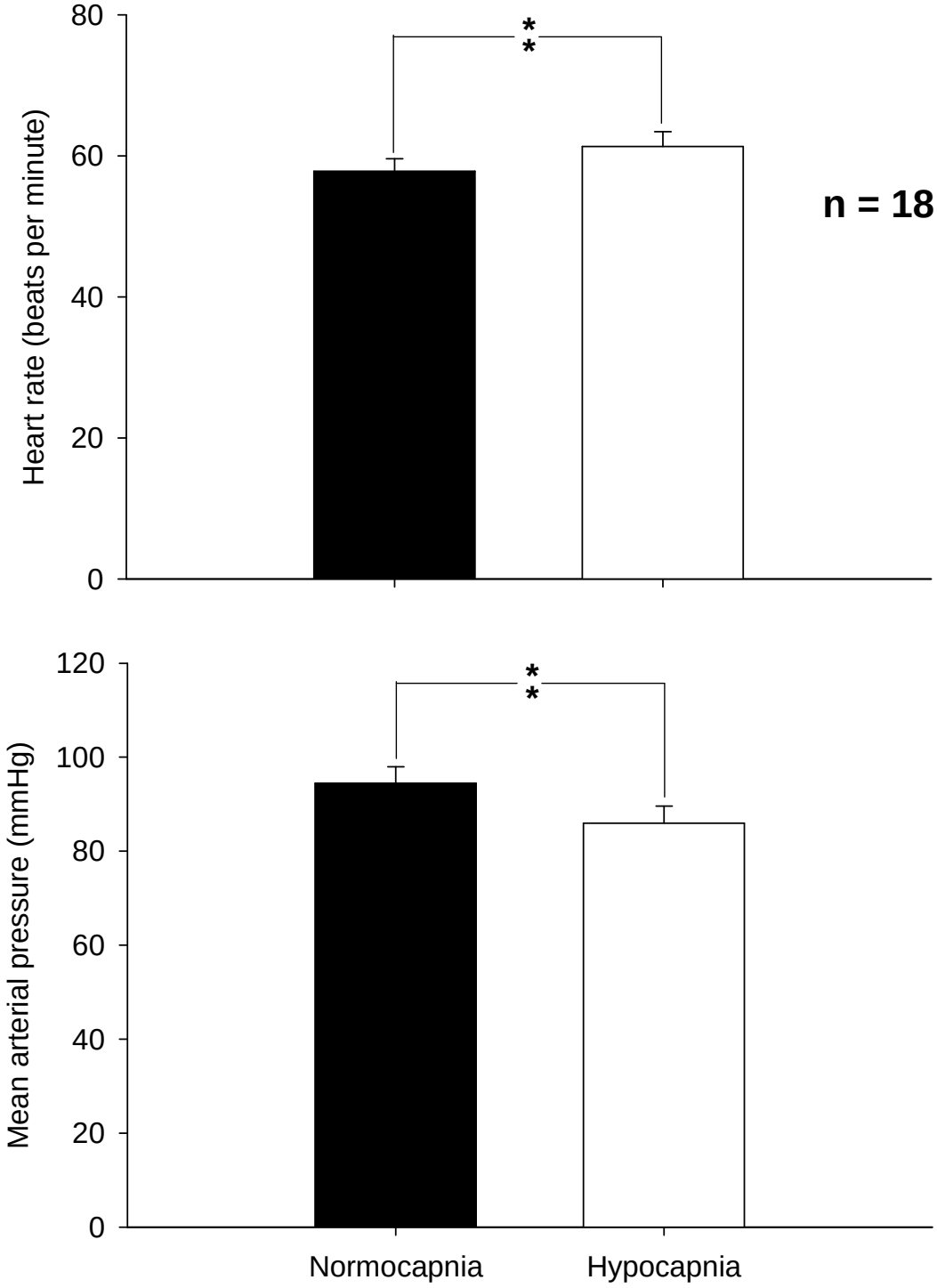


Figure 4.5. Mean ± SE heart rate and blood pressure changes during mechanical hyperventilation in normocapnia and hypocapnia in 18 subjects. ** *P* < 0.01 vs normocapnia.

4.4.1. Electrocardiography

During hypocapnia, a reduction in T wave amplitude occurred in 11/18 subjects (61%) in lead I of the ECG. The largest decrease in any one subject was 0.05mV (decrease of 14%). However, the mean decrease in T wave amplitude was just 0.002 ± 0.025mV (decrease of 1%) and this was not statistically significant ($p \geq 0.80$) (figure 4.6). As expected, there was no significant change in R wave amplitude in lead I (0.49 ± 0.05mV [normocapnia] vs. 0.49 ± 0.05mV [hypocapnia] [$p > 0.95$]) (figure 4.7).

Hypocapnia caused a statistically significant increase in T wave amplitude in the anteroseptal transverse plane leads; V_1 (increase of 0.06 ± 0.01mV [> 31%], $P < 0.01$), V_2 (increase of 0.09 ± 0.02mV [> 15%], $P < 0.001$) and V_3 (increase of 0.07 ± 0.02mV [> 11%], $P < 0.05$) (figure 4.6). These changes were not accompanied by any R wave changes (figure 4.7) and were within clinically acceptable limits for normal T wave amplitudes (<1.0mV). Increases in T wave amplitude were not accompanied by significant changes in the ST segment in any of the 12 leads of the ECG (figure 4.8). The largest ST segment deviation was seen in V_4 (increase of 0.02 ± 0.01mV, $P > 0.20$) but this was within clinically acceptable limits.

When T wave and ST segment data in the transverse plane was separated by gender, increases in T wave amplitude persisted in lead V_1 (0.06 ± 0.02mV [> 40%] in men and 0.06 ± 0.02mV [> 125%] in women, $P <0.05$) (figure 4.9). In addition, statistically significant increases in T wave amplitude persisted in leads V_2 (0.10 ± 0.02mV [15%], $P < 0.01$) and V_3 (0.08 ± 0.02mV [11%], $P < 0.05$) in male subjects but disappeared in female subjects (figure 4.9). T wave amplitudes in both men and women were below their respective gender specific clinical thresholds.

Figure 4.6. Hypocapnia does not significantly decrease T wave amplitude in any of the 12 leads of the ECG but does cause significant T wave elevation in the antero-septal leads V_{1-3}

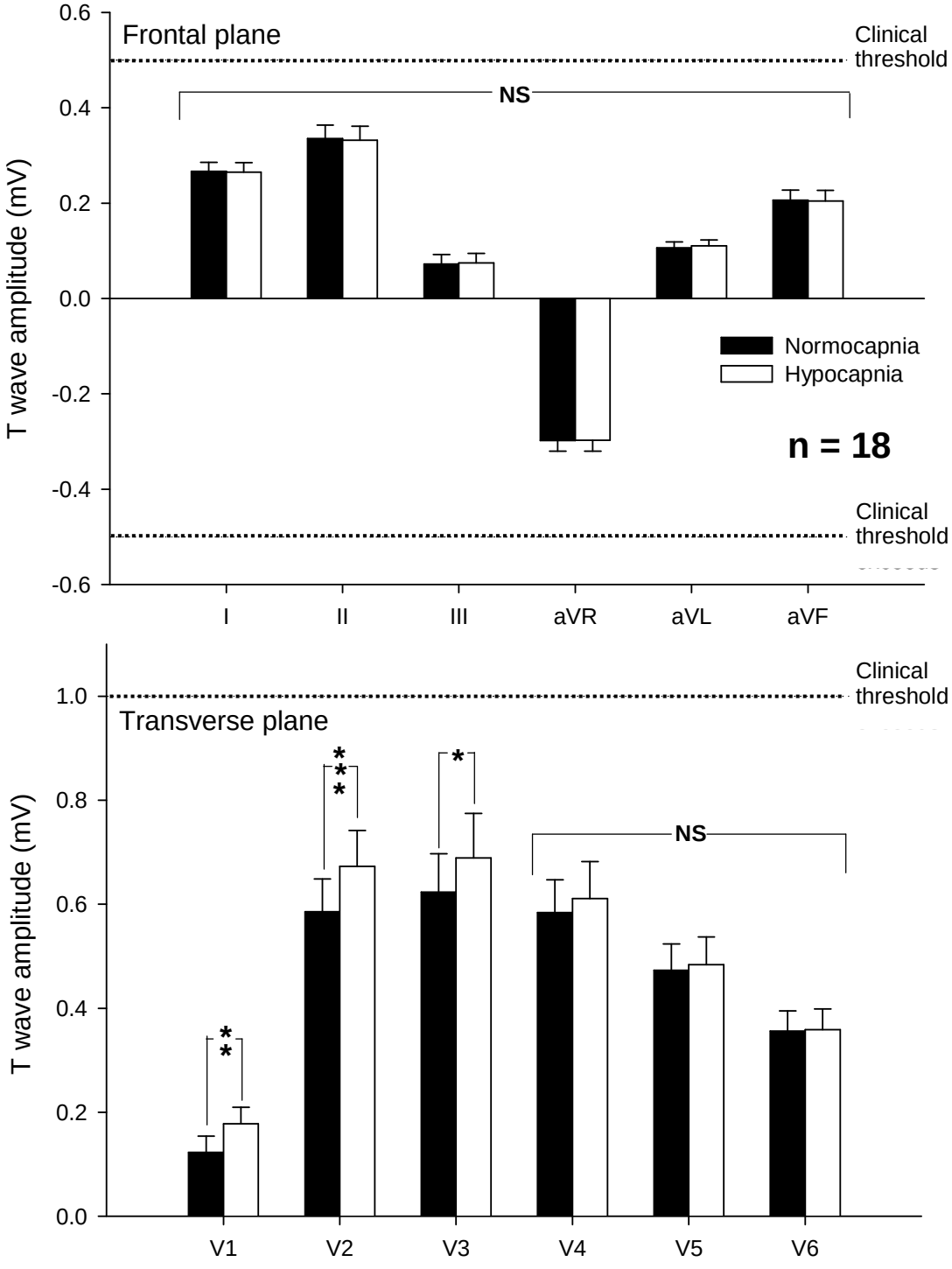


Figure 4.6. Mean ± SE T wave amplitudes during mechanical hyperventilation in normocapnia and hypocapnia. * *P* < 0.05, ** *P* < 0.01, * *P* < 0.001 and NS vs. normocapnia. Dotted lines indicate upper thresholds for normal T wave amplitudes.**

Figure 4.7. Hypocapnia does not have any significant affect on R wave amplitude in any of the 12 leads of the ECG in 18 normal subjects

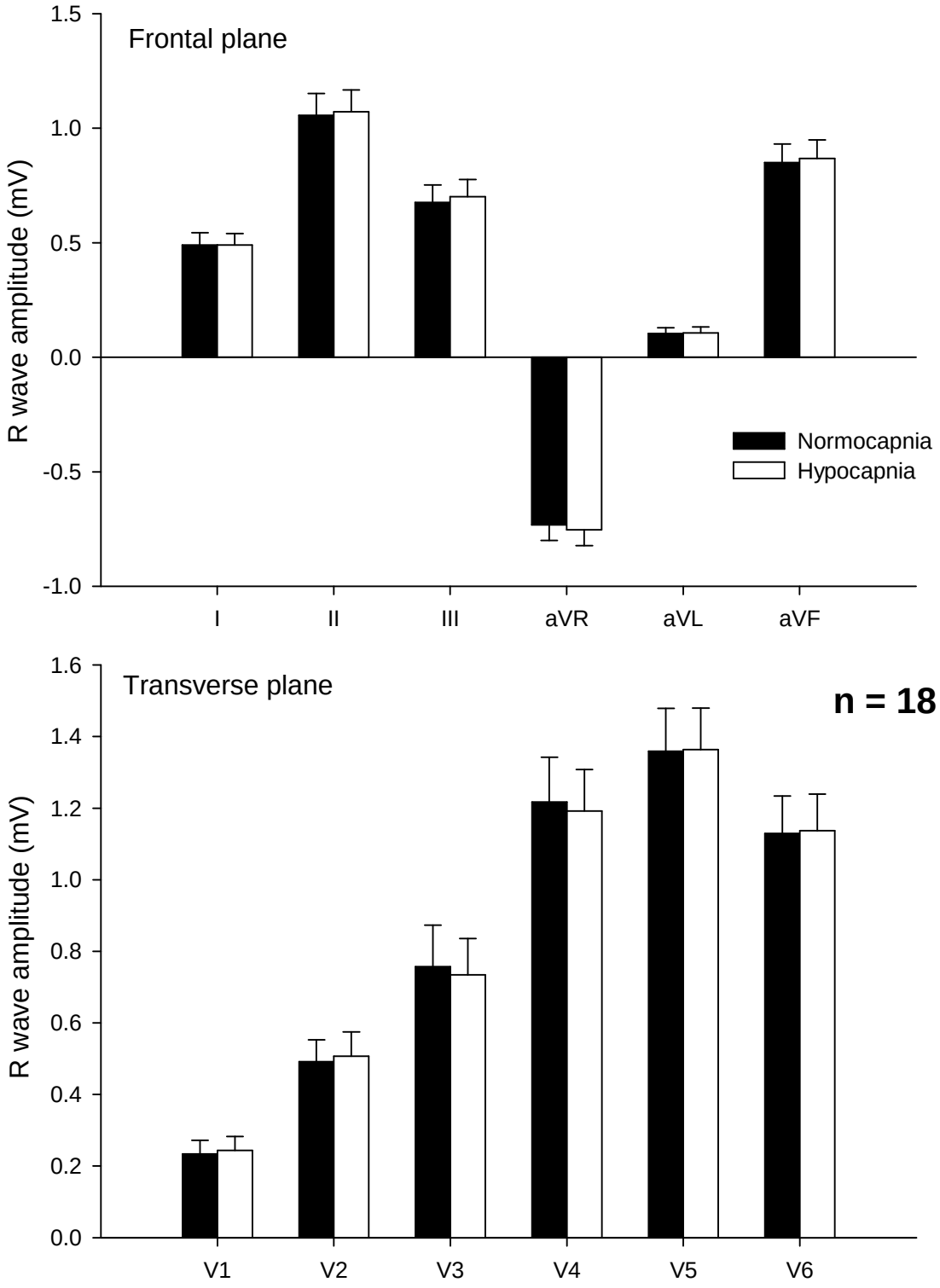


Figure 4.7. Mean ± SE R wave amplitudes during mechanical hyperventilation in normocapnia and hypocapnia. All wave amplitudes in hypocapnia, *NS* vs. normocapnia

Figure 4.8. Hypocapnia did not cause significant ST segment changes in any of the 12 leads of the ECG during mechanical hyperventilation

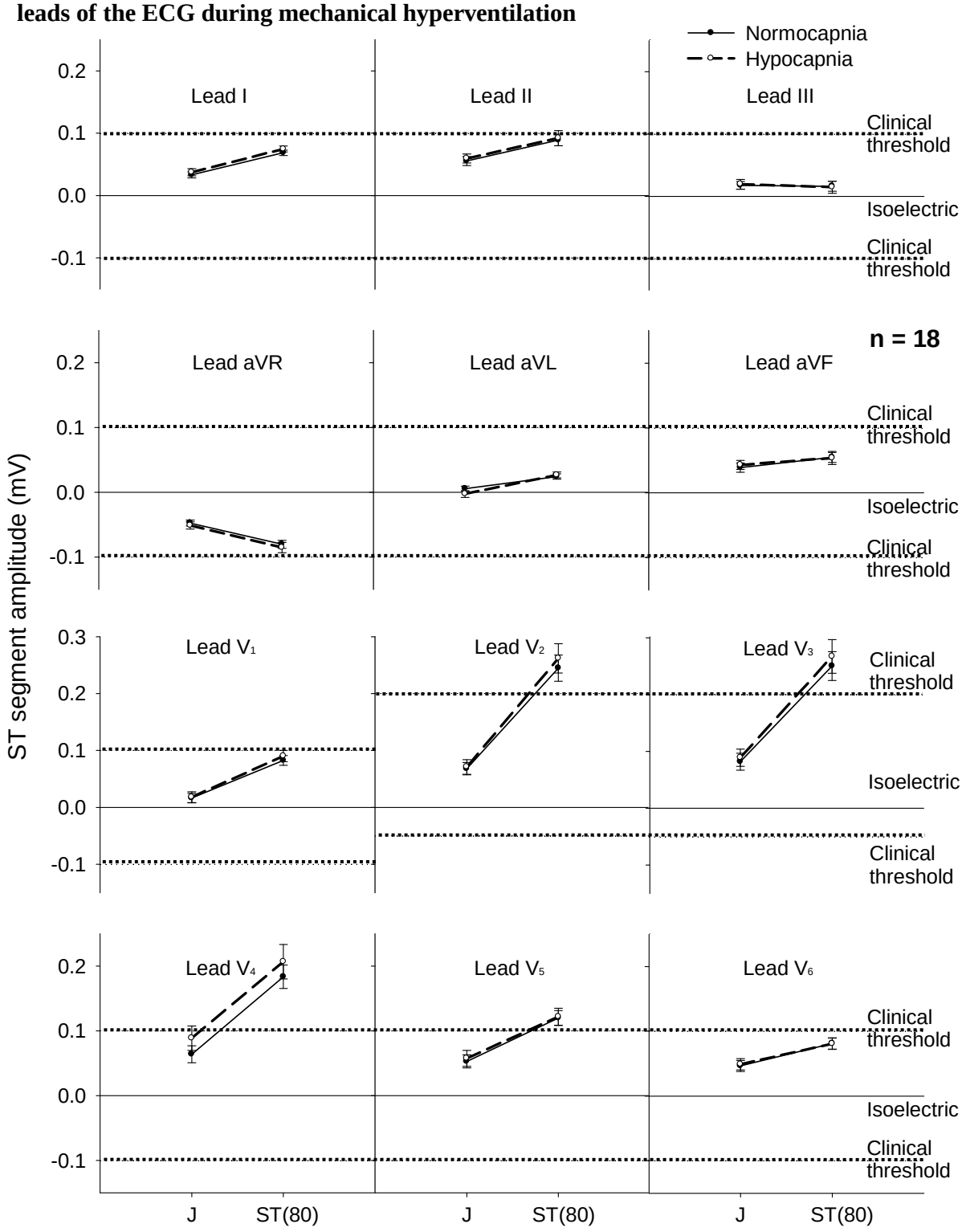


Figure 4.8. Mean ± SE ST segment height, defined by deviation of the J point from the isoelectric line. The extent of these changes is estimated by deviation of the ST(80) point. All points in hypocapnia, NS vs. normocapnia. Dotted line represents clinically significant deviation from isoelectric line.

Figure 4.9. Hypocapnia causes T wave elevation in leads V_{1-3} in male subjects but only causes T wave elevation in V_1 in female subjects

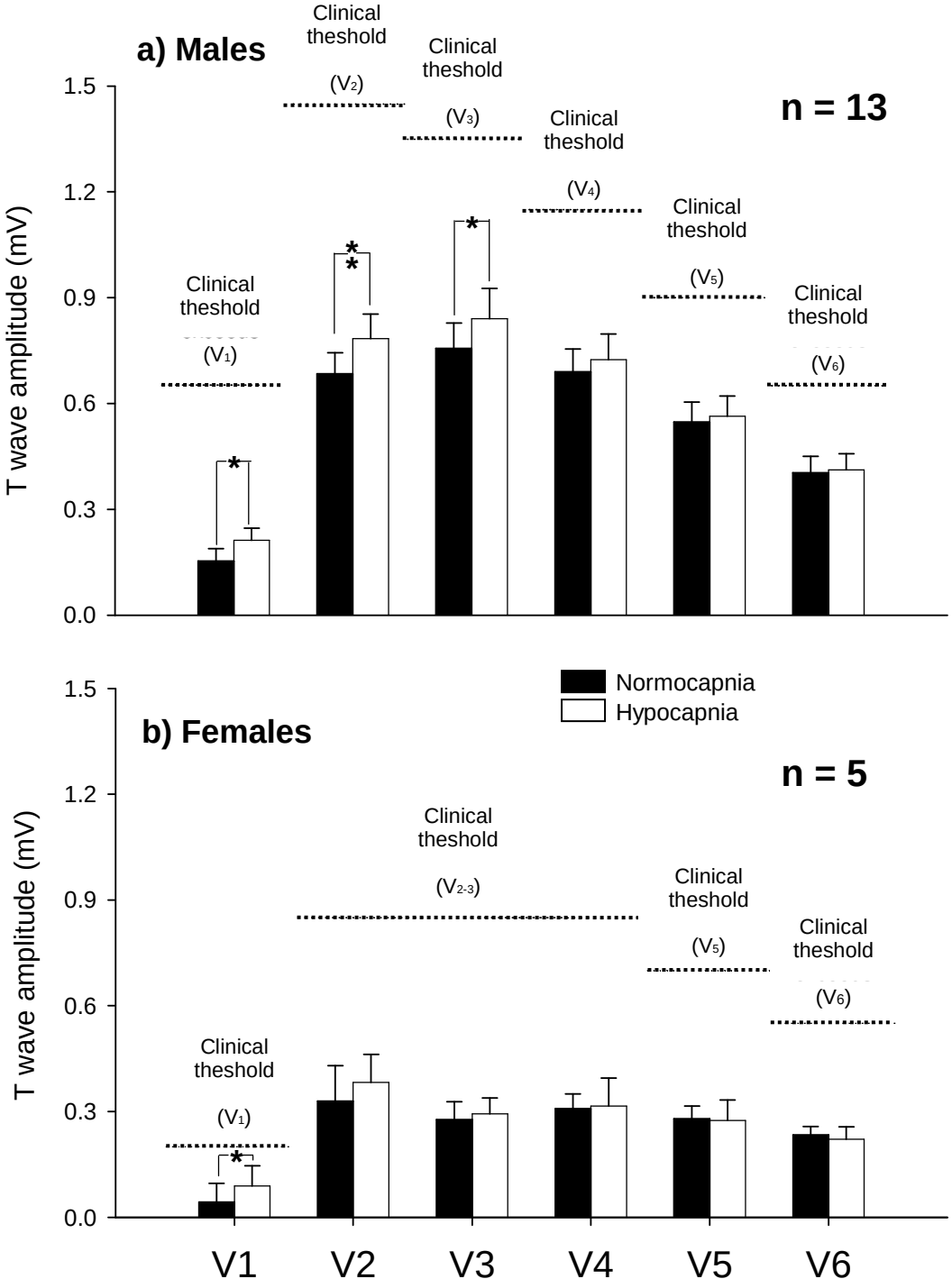


Figure 4.9. Mean ± SE T wave amplitude in the transverse plane during normocapnia and hypocapnia in males (a) and females (b). Gender specific clinical thresholds for abnormality (.....) in the T wave (table 2.4) are also shown. All amplitudes in hypocapnia, * $P < 0.05$, ** $P < 0.01$ or *NS* vs. normcapnia.

ST segment height in the transverse plane of female subjects was unaffected by hypocapnia (figure 4.10b). In male subjects, hypocapnia caused statistically significant ST segment elevation in lead V_3 (0.02 ± 0.01mV [22%], $P < 0.05$) but this was not accompanied by significant ST(80) point elevation and was below the clinical threshold for normality (figure 4.10a). In lead V_4 in male subjects, the ST segment was elevated beyond clinical limits for normality during hypocapnia but this was not statistically significant (0.04 ± 0.02mV [57%], $P > 0.05$) (figure 4.10a).

In all subjects, hypocapnia did not cause statistically or clinically significant QTc interval prolongation in any of the 12 leads of the ECG (figure 4.11).

Figure 4.10. Hypocapnia causes significant ST segment elevation in lead V_3 in 13 normal male subjects but not in 5 female subjects when data is seperated by gender

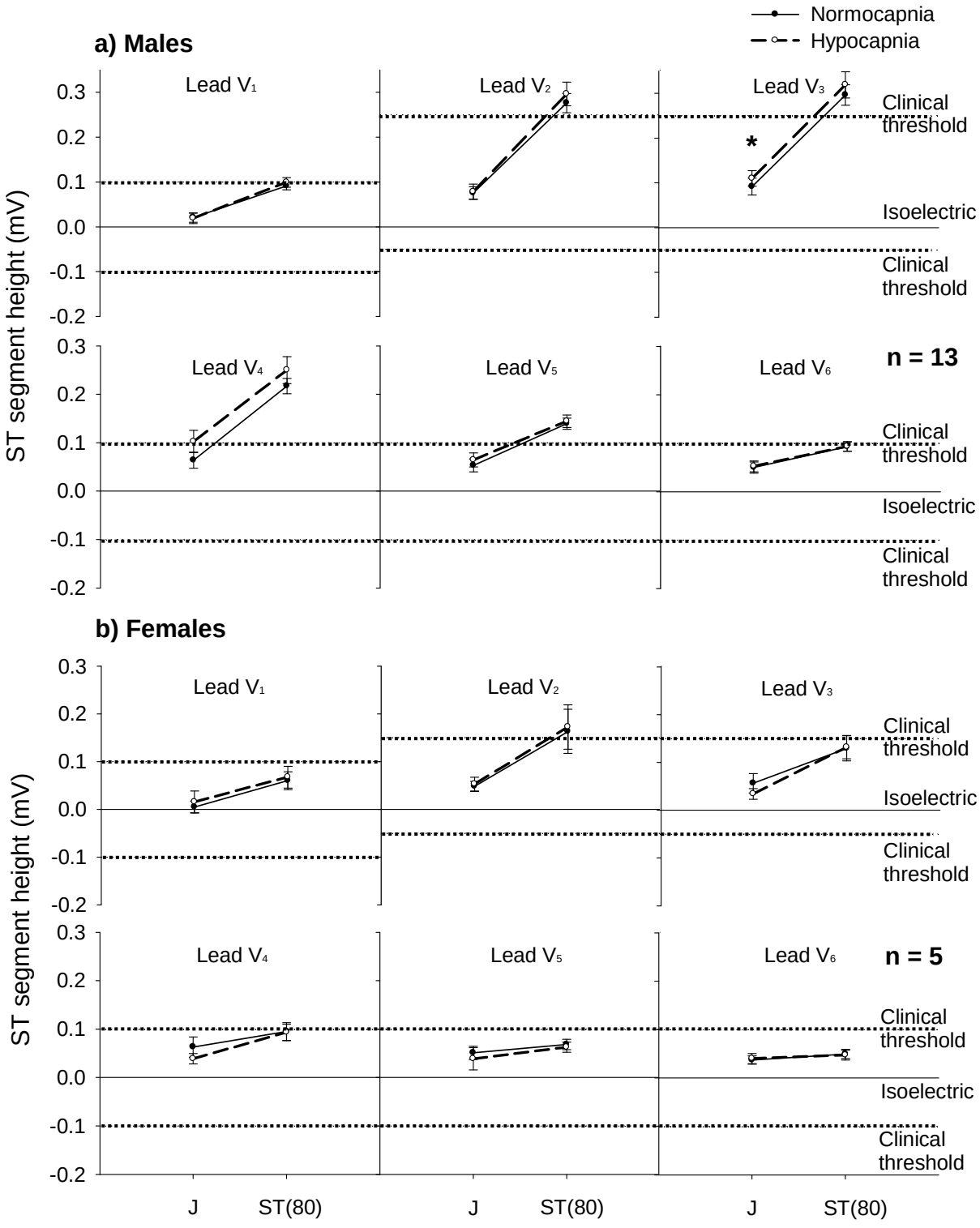


Figure 4.10. Mean ± SE of ST segment height (J point & ST[80] point) in the transverse plane in males (a) and females (b). Dotted lines are gender sepecific and represent clinically significant deviation from isoelectric line (straight line). All ST heights in hypocapnia, * *P* < 0.05 or *NS* vs. normocapnia.

Figure 4.11. Hypocapnia does not cause significant QTc interval prolongation in any of the 12 leads of the ECG of 18 normal subjects

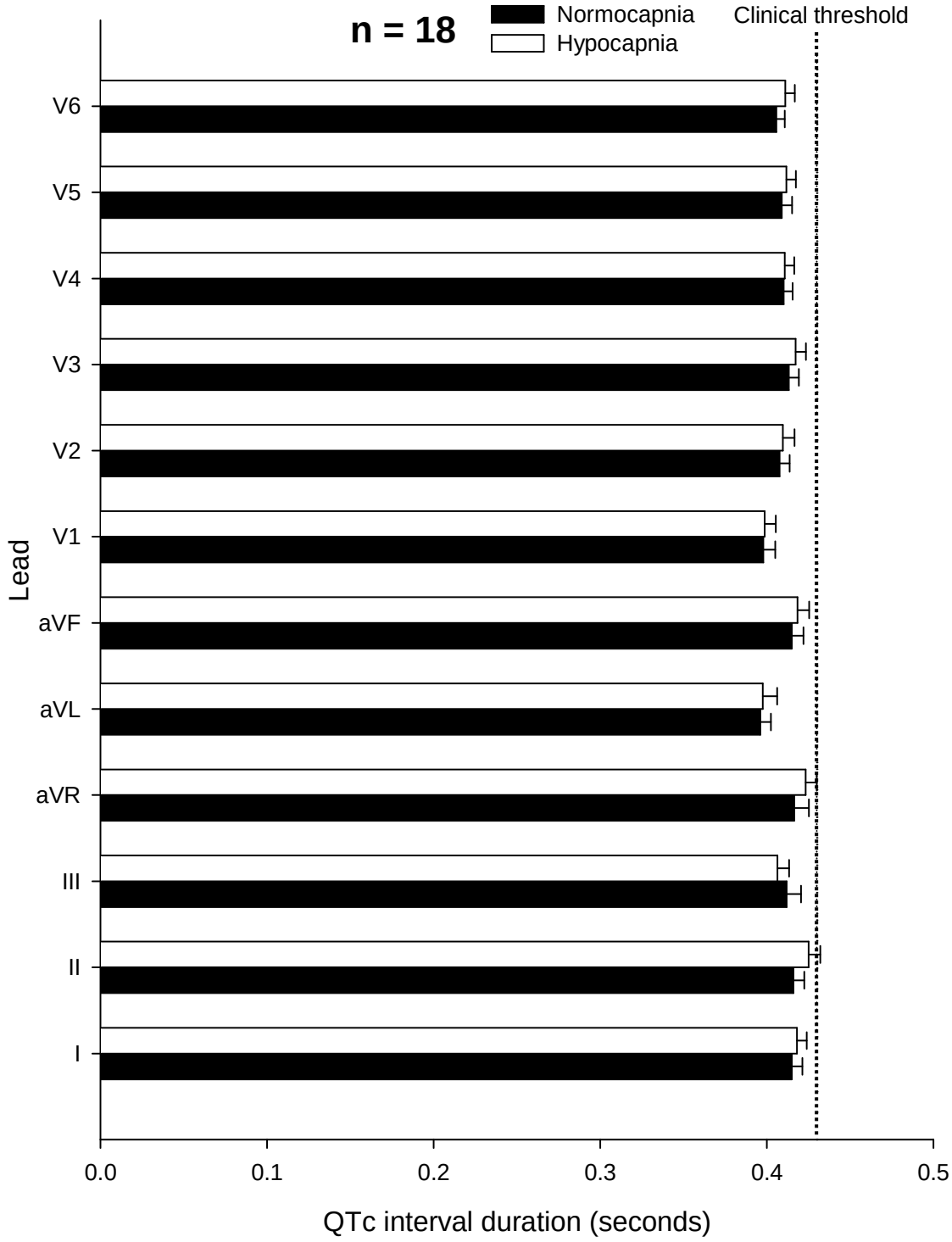


Figure 4.11. Mean ± SE QTc interval duration during mechanical hyperventilation in normocapnia and hypocapnia. All durations in hypocapnia, *NS* vs. normocapnia. The dotted line indicates the upper threshold for normal QTc interval duration.

4.4.2. Echocardiography

Figure 4.12 shows that hypocapnia has no effect on (a) LVID difference or (b) fractional shortening during mechanical hyperventilation, measured by m-mode echocardiography in 18 subjects. LVID difference (-1.8 ± 0.0cm) and fractional shortening (34%) during hypocapnia were within clinically normal limits. This indicates that left ventricular contractility was not affected by hypocapnia as the left ventricle walls contracted to the same degree in both normocapnia and hypocapnia.

Doppler blood flow analysis performed on the left ventricle at the level of the mitral valve revealed no evidence of diastolic dysfunction during hypocapnia. A non significant decrease in E/A ratio from 1.99 ± 0.13 in normocapnia to 1.78 ± 0.14 was recorded during hypocapnia ($P \geq 0.10$) (figure 4.13a) but this was accompanied by a normal deceleration time (0.20 ± 0.01 seconds) (figure 4.13b). These values were within clinically normal limits (E/A ratio >1 and deceleration time <0.22).

Tissue Doppler analysis of septal and lateral wall velocity revealed no evidence of myocardial ischemia. Figure 4.14 shows that during hypocapnia, diastolic wall velocities in both the septum and lateral wall remained unchanged. Septal wall velocity (12.6 ± 0.5cm/s) (figure 4.14a) and lateral wall velocity (16.7 ± 0.6cm/s) (figure 4.14b) during diastole were not significantly different in hypocapnia from normocapnia ($P > 0.60$) and were comparable with clinically normal values.

Figure 4.12. Hypocapnia has no affect on LVID difference (a) or fractional shortening (b) measured by m-mode echocardiography

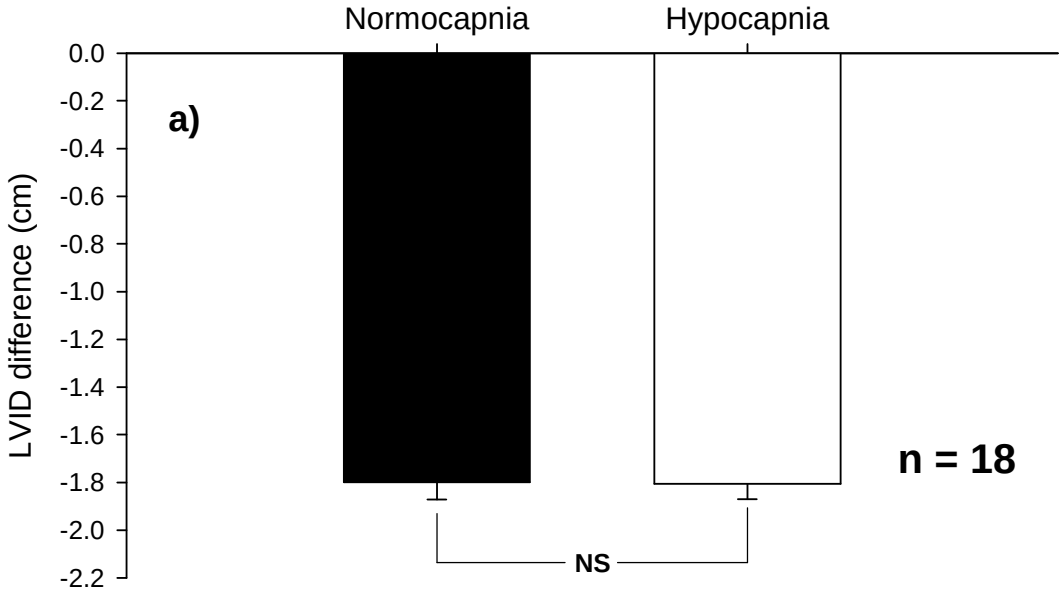


Figure 4.12a. Mean ± SE LVID difference is calculated as the difference between LVID in diastole and systole. Values in hypocapnia, *NS* vs. normocapnia

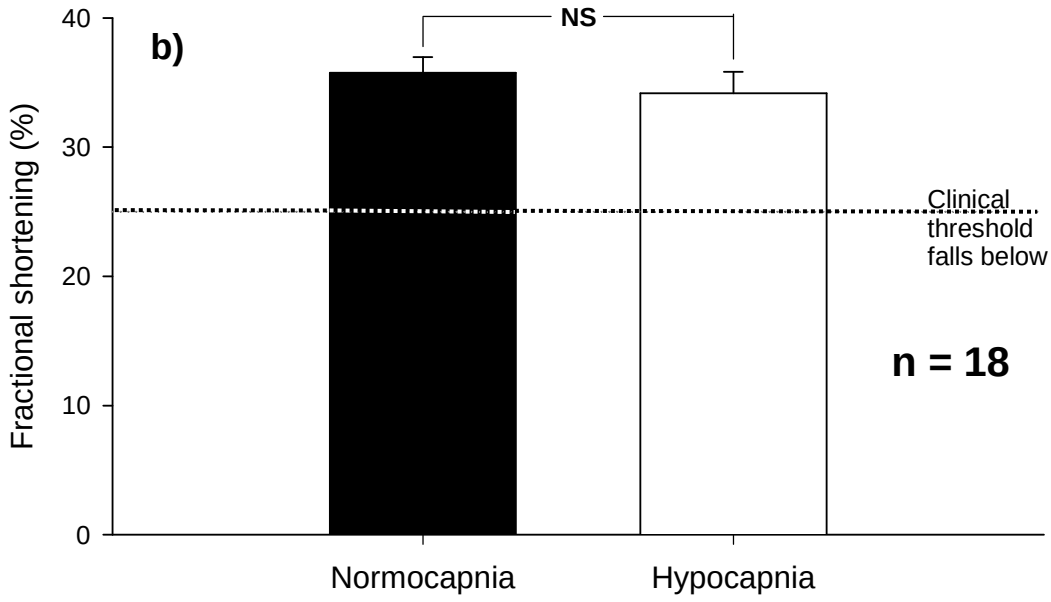


Figure 4.12b. Mean ± SE Fractional shortening during normocapnia and hypocapnia. Values in hypocapnia, *NS* vs. normocapnia. Dotted line indicates the lower clinical threshold for normal fractional shortening.

Figure 4.13. Hypocapnia causes no significant decrease in E/A ratio (a) and deceleration time (b) measured by Doppler blood flow echocardiography

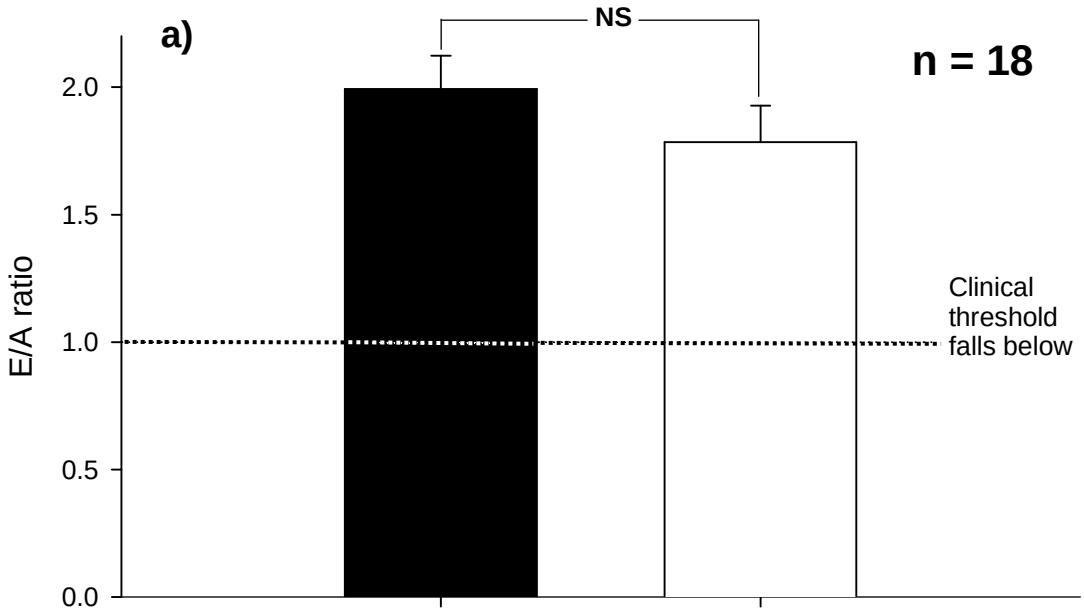


Figure 4.13a. Mean ± SE E/A ratio measured from the Doppler trace during mechanical hyperventilation normocapnia and hypocapnia. Values in hypocapnia, *NS* vs. normocapnia. Dotted line indicates the lower threshold for normal ratios.

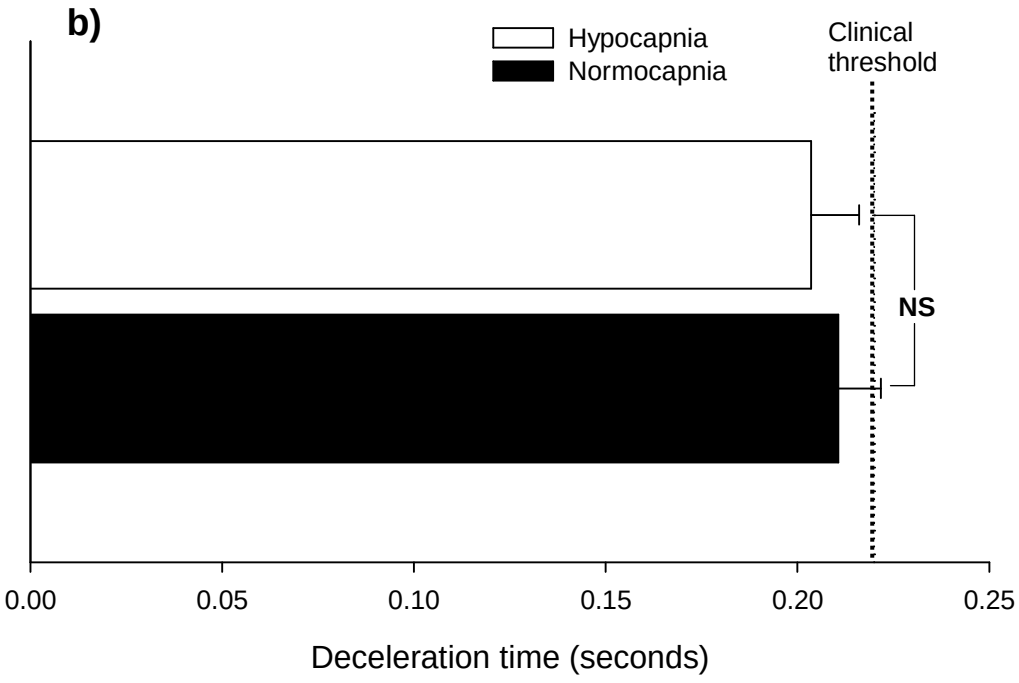


Figure 4.13b. Mean ± SE Deceleration time measured from the Doppler trace during mechanical hyperventilation in normocapnia and hypocapnia. Values in hypocapnia, *NS* vs. normocapnia. Dotted line indicates the upper clinical threshold for normal durations.

Figure 4.16. Hypocapnia causes no changes in diastolic wall velocity in either the septum (a) or lateral wall (b) measured by tissue Doppler echocardiography

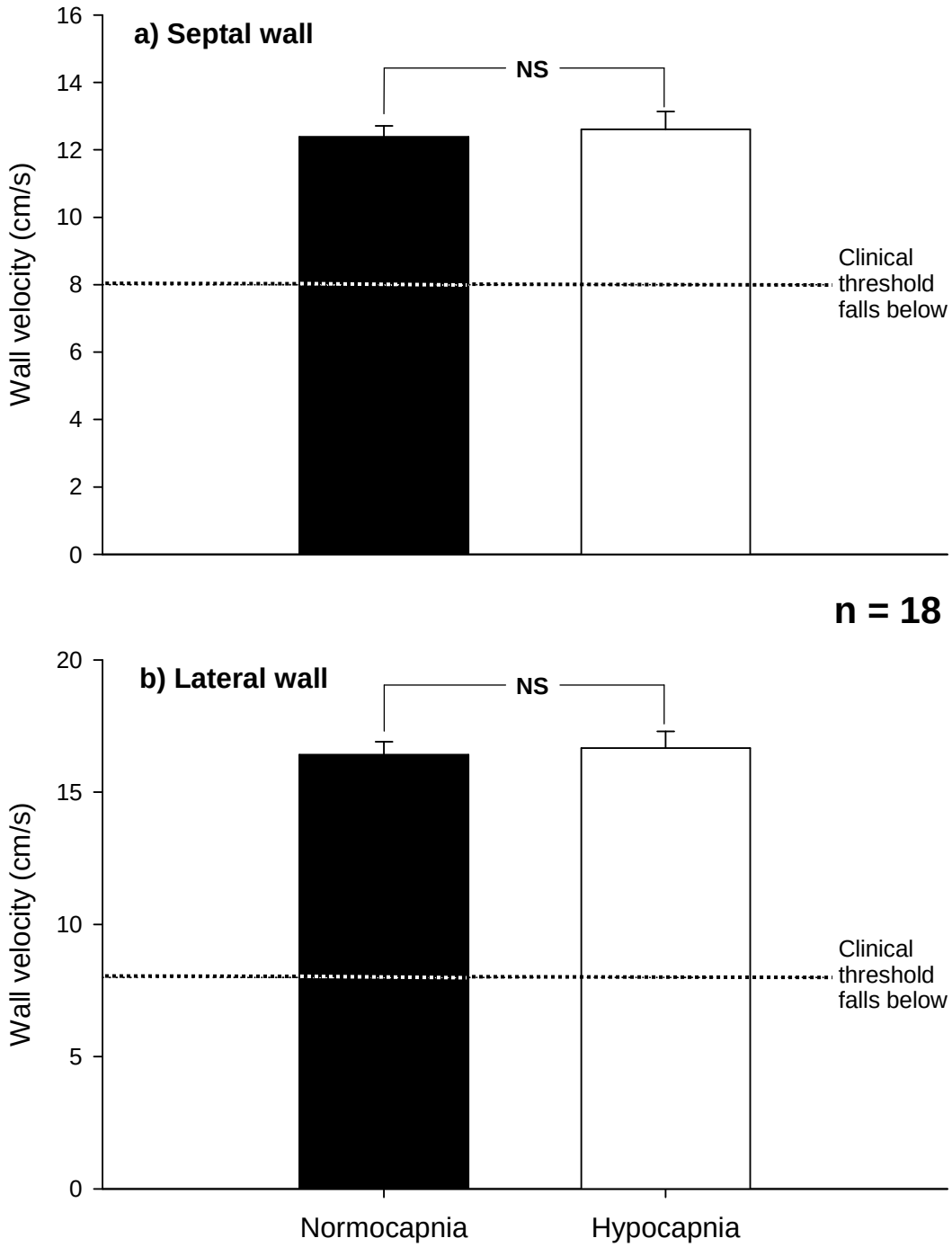


Figure 4.16. Mean ± SE Septal and lateral wall velocity in the left ventricle during diastole in normocapnia and hypocapnia measured from the peak of the E wave on the tissue Doppler trace. Values in hypocapnia, *NS* vs. normocapnia. Dotted line indicates the lower clinical threshold for normal velocities.

4.5. Discussion

The present study shows that the most severe hypocapnia (20 ± 0mmHg) tolerable by normal subjects does not induce clinically significant changes on the 12 lead ECG or echocardiogram. These findings suggest that even though a more stable and sustained period of hypocapnia was induced than in previous studies using voluntary hyperventilation (table 1.2, page 19), it was still not sufficient to reduce O_2 delivery to the myocardium to such a degree that noticeable changes in cardiac electrical activity or heart function could be recorded.

4.5.1 Changes in heart rate & blood pressure

Mechanical hyperventilation, without the addition of CO_2 to inspired air, caused $PetCO_2$ to decrease by 18 ± 0mmHg to 20mmHg which caused blood pH to increase to 7.6 ± 0.0 pH units. Hypocapnia caused a small but significant increase in heart rate (4 ± 1 bpm). These findings confirm previous studies that hypocapnia causes only trivial changes in heart rate during mechanical hyperventilation (Rutherford *et al.*, 2005; Prys-Roberts *et al.*, 1972; Cooper *et al.*, 2004; Kazmaier *et al.*, 1998). These small changes are thought to be caused by aortic chemoreceptor stimulation caused by a hypocapnia induced decrease in O_2 availability in arterial blood (Rutherford *et al.*, 2005). In the present study, the fact that few significant ECG or echocardiographic changes occurred during hypocapnia, would suggest that such heart rate changes do not have any significant effect on the ECG waveform.

In contrast, previous studies with voluntary hyperventilation have reported increases of 9-58 bpm (Biberman *et al.*, 1971; Christensen, 1946; Joy & Trump, 1981; Mcgregor *et al.*, 1962; Miyagi *et al.*, 1989; Previtali *et al.*, 1989; Richardson *et al.*, 1972; Burnum *et al.*, 1954)

during hypocapnia. It is most likely that these cardiovascular changes occurred due to the physical exertion associated with voluntary hyperventilation.

Hypocapnia caused a statistically significant decrease in mean arterial blood pressure (9 ± 3mmHg). Previous studies report non-significant decreases of up to 7mmHg (Cooper *et al.*, 2004; Prys-Roberts *et al.*, 1972) and no change (Kazmaier *et al.*, 1998; Rutherford *et al.*, 2005) in mean arterial pressure during hypocapnia induced by mechanical hyperventilation. It is unclear whether the drop in blood pressure seen during the present investigation represents a true physiological effect of hypocapnia or just a limitation of the Finapres device for accurately measuring changes in blood pressure. It has been shown that the Finapres can overestimate absolute blood pressure values by up to 7 ± 11mmHg (Wesseling *et al.*, 1985; Imholz *et al.*, 1988; van Egmond *et al.*, 1985; 1990). An overestimation of this amount would cause the size any observed blood pressure change to be exaggerated. In addition, despite attempts to prevent this from occurring, it is possible that the subjects' hands may have become cold during the experiment, resulting in a reduction in blood flow to the fingers. This would limit the accuracy of subsequent estimations of blood pressure by the Finapres. Due to the lack of supporting evidence for a reduction of blood pressure during mechanical hyperventilation, the lack of electrocardiographic and echocardiographic changes and the limitations of the Finapres device used, the changes in blood pressure seen in the present study were not thought to be of physiological importance for the findings of this study.

4.5.2. T wave changes during hypocapnia

Hypocapnia caused a small non-significant reduction in T wave amplitude in 61% of subjects in lead I of the ECG. These findings were much smaller than those of Rutherford *et al.*,

(2005) who demonstrated a significant T wave reduction of 0.1mV in 87% of subjects during hypocapnia induced by mechanical hyperventilation. The discrepancies between the Rutherford *et al.,* (2005) study and the present investigation appear to be caused by the different ECG apparatus used. In the study by Rutherford *et al.,* (2005), a single lead ECG was recorded using an analogue Neurolog NL840 amplifier calibrated against a custom built 200uV generator. In contrast, the ECG apparatus used in the present study was calibrated against a patient simulator calibrator box (Dynatech Nevada Inc., Model 212B, Nevada, USA) and a standard digital ECG machine (Philips Hewlett Packard Pagewriter 200) commonly used in clinical practice. T wave amplitudes at rest in the Rutherford *et al.,* (2005) study were 0.73mV, which is more than double the size of those in the present study (0.32mV). The resting T wave amplitudes in the Rutherford *et al.,* (2005) study would be considered clinically abnormal (hyperacute) if they were compared to the clinical thresholds used in the present study (>0.5mV [Wagner, 2008]). Because the data recorded in the present investigation were analogous to clinical ECG machines, they were considered comparable to clinical thresholds set out by the AHA, ACCF, HRS (Rautaharju *et al.,* 2009; Surawicz *et al.,* 2009; Wagner *et al.,* 2009) and Wagner (2008).

Hypocapnia, induced by mechanical hyperventilation, caused significant T wave changes in the anteroseptal (transverse plane) leads V_{1-3} (increase of up to 0.09mV, 31%). These changes were not accompanied by any significant changes in the ST segment or QTc interval. In addition, these increases in T wave amplitude did not cause hyperacute T waves with amplitudes beyond non-gender specific clinical thresholds for normality. When T wave and ST segment data in the transverse plane were separated by gender, increases in T wave amplitude in the anteroseptal leads persisted in the male group. In females, significant

increases in T wave amplitude persisted in lead V_1 but disappeared in leads V_{2-3}. Importantly, in neither group did T wave amplitudes increase beyond gender specific clinical thresholds for normality.

It is unlikely that T wave changes in the anteroseptal leads were caused by changes in plasma electrolytes. It is thought that voluntary hyperventilation causes increases (of 0.3-0.5mEq/L from 3.6-4.6mEq/L) (Yu *et al.*, 1959; Biberman *et al.*, 1971; Krapf *et al.*, 1995) or decreases in blood potassium (of 0.2mEq/L from 4mEq/L) (Mostellar & Tuttle, 1964) and decreases in blood calcium (of 0.1mg/100ml from 10-10.5 ± 0.1mg/100ml) (Yu *et al.*, 1959). In the present investigation, no changes in ionized potassium or electrocardiographic indicators of hypokalemia or hypocalcemia were observed. Significant decreases in ionized calcium did occur during hypocapnia (-0.03 ± 0.00mmol/l, $P < 0.001$), however, absolute ionized calcium concentrations (1.17 ± 0.00mmol/l) were within the clinically normal range (1.03-1.23mmol/l, Larsson & Ohman, 1978) and therefore were unlikely to cause ECG changes.

The use of mechanical hyperventilation to induce hypocapnia enabled inflation volume to be kept constant throughout the experiment. Therefore, increases in T wave amplitude in the anteroseptal leads could not have been caused by changes in inflation volume.

It is possible that increases in T wave amplitude observed in the present study represents the very earliest signs of grade I (early) localised transmural ischemia, caused by a decrease in O_2 availability in the myocardium due to increased coronary vasoconstriction and a decrease in O_2 dissociation in the anteroseptal region of the heart (Rowe *et al.*, Karlsson *et al.*, 1994; Laffey & Kavanagh, 2002; Neill & Hattenhauer, 1975; 1962). Alternatively, increases in T

wave amplitude may have occurred due to normal variation of the T wave, known to occur in normal resting subjects (Taggart *et al.*, 1979). It is difficult to draw conclusions as to the origins of the T wave changes seen in the present study because;

- T wave changes seen in the anteroseptal leads (V_{1-3}) were within normal clinically acceptable limits for healthy subjects (<1mV) (Wagner, 2008) and were not accompanied by any other ECG changes.
- T wave changes alone are not reliable indicators of ischemia because clinically significant T wave changes occur in normal healthy subjects with normal coronary arteriograms (Taggart *et al.*, 1979) and sometimes fail to appear in patients suffering from myocardial ischemia (Wagner, 2008).
- Hyperacute T waves represent transmural ischemia in a region of the heart due to decreased blood supply caused by total coronary occlusion (Wagner, 2008). It is unlikely that total occlusion would occur as a result of hypocapnia in subjects with normal healthy coronary arteries, particularly in the absence of other markers for early transmural ischemia such as QTc interval prolongation (Kenigsberg *et al.*, 2007).

4.5.3. Hypocapnia and echocardiography

Non-invasive echocardiography was employed as an independent measure of heart function during hypocapnia. No detectable nor statistically significant change in LIVD difference, fractional shortening, or tissue Doppler myocardial wall velocities were seen during hypocapnia in any subject. This suggests that hypocapnia had no effect on left ventricular wall motion during mechanical hyperventilation. There was also no decrease in E/A ratio or deceleration time measured during hypocapnia using Doppler blood flow echocardiography.

This suggests that hypocapnia did not induce myocardial ischemia of sufficient severity to stimulate a detectable reduction in global left ventricular function.

4.5.4. Conclusions

Indirect measures of cardiac electrical activity and heart function were not able to detect a noticeable ischemic effect of hypocapnia in normal healthy subjects. It has been calculated that the coronary arteries need to be occluded by at least 30-60% before blood flow is significantly impaired (May *et al.,* 1963; Braunwald *et al.,* 2001). Whilst it appears that hypocapnia does not induce a critical stenosis (30-60% occlusion) sufficient enough to reduce blood flow and cause ischemic ECG changes in normal healthy subjects, it remains to be seen whether ECG changes can be induced in patients already suffering from restricted coronary blood flow due to coronary artery disease.

The calculations by May *et al.,* (1963) and Braunwald *et al.,* (2001) do not consider the additional effects of hypocapnia on the affinity of haemaglobin for O_2. It is of interest to investigate whether the ECG changes that were observed during hypocapnia in normal subjects can be accentuated by hyperventilation in 15% O_2 where myocardial O_2 availability should be further reduced.

Chapter 5

Can mechanical hyperventilation in 15% inspired O_2 induce ischemic ECG changes in normal subjects?

5.1. Summary

The aim of this investigation was to see whether hypocapnia induced by mechanical hyperventilation in 15% O_2 can provoke consistent ischemic ECG changes in normal subjects. This level of inspired O_2 was considered safe for use in a clinical setting because it is equivalent to the air inspired on commercial aircraft and is not thought to cause chest pain or discomfort at rest.

Spontaneous breathing with 15% O_2 significantly reduced O_2 saturation to 93 ± 1% (P < 0.001) but otherwise had no affect on heart rate, blood pressure or ventilation. Hypocapnia in 15% O_2 did not significantly augment ECG changes seen during hypocapnia in 21% O_2 or induce clinically significant ischemic changes in the R and T wave, ST segment or QTc interval.

These findings show that hypocapnia induced by mechanical hyperventilation in 15% inspired O_2 does not augment or induce significant ischemic ECG changes in normal healthy subjects, despite presumably inducing a more severe reduction in O_2 availability than during hypocapnic mechanical hyperventilation in 21% O_2. The fact that mechanical hyperventilation in 15% inspired O_2 did not accentuate the increase in T wave amplitude seen during hypocapnia in 21% O_2 suggests that these T wave changes were not caused by a decrease in

oxygen delivery to the heart. This indicates that in normal subjects, hypocapnia of this severity (20mmHg) is not sufficient to induce the critical stenosis necessary to substantially reduce blood flow and O_2 delivery to the heart.

5.2. Introduction

Hypoxemia refers to an abnormally low O_2 content in arterial blood (Steadman, 1972). It can be induced by inhaling 7.5-12% O_2 for 5-35 minutes and causes T wave depression/flattening of up to 0.3mV and ST changes in normal subjects (Broch, 1972b; Katz *et al.*, 1934; Levy *et al.*, 1938; May, 1939; Levy *et al.*, 1939; Barach *et al.*, 1941; Haarstad & Broch, 1958). In patients with coronary artery disease, hypoxemia stimulates T wave depression (up to 0.65mV), T wave inversion (< -0.15mV), ST changes (> 0.1mV) and chest pain (Broch, 1972b; Katz *et al.*, 1934; Levy *et al.*, 1938; Barach *et al.*, 1941; 1939; 1941; Turner & Morton, 1952; Haarstad & Broch, 1958; Rothschild & Kissin, 1933). Because these effects are more pronounced in patients, the hypoxemia test was proposed as a provocative test for coronary artery disease (Rothschild & Kissin, 1933; Levy *et al.*, 1938; 1939; 1941; Turner & Morton, 1952; Haarstad & Broch, 1958; Broch, 1972a; 1972b; Stewart & Carr, 1954).

Levy *et al.*, (1938; 1939) standardised the hypoxemia test with a specific protocol involving inspiration of a 10% oxygen in 90% nitrogen gas mixture for 20 minutes whilst the ECG (leads I-III and a precordial lead called IVF) was recorded before, during and after the test. A criterion of threshold ECG values was developed for assessing coronary efficiency with a 'positive' response to the test indicating the existence of coronary artery disease. An abnormal response to hypoxia (a positive hypoxemia test) was defined by ST deviation in all four leads

(I-III, IVF) totalling >0.3mV, inverted T waves in lead I coupled with a ST change of >0.1mV and/or T wave depression in lead IVF with ST deviation of >0.1mV (Levy *et al.*, 1939; 1941).

In patients with known coronary artery disease, the sensitivity and specificity of the hypoxemia test (using the Levy diagnostic criteria) is between 30-79% and 86-100% respectively (Levy *et al.*, 1941; Turner & Morton, 1952; Haarstad & Broch, 1958; Stewart & Carr, 1954). However, the existence of coronary artery disease was identified by previous patient history and previous ECG records. The hypoxemia test has not been compared to a recognised gold standard (*e.g.* coronary angiography) and thus these sensitivity and specificity must be considered with caution.

The hypoxemia test has a number of limitations;

1) The hypoxemia test causes patient distress. Rothschild & Kissin, (1933) showed that 69% of patients with coronary artery disease, chest pain developed during the hypoxemia test and on a number of these occasions (number of occasions not specified), the test had to be stopped early. Extraordinarily, the authors described the appearance of chest pain and discomfort as *"an evil necessary for the successful performance of the test"*. Studies by Levy *et al.,* (1938; 1939; 1941), Barach *et al.,* (1941), Turner & Morton (1952) and Haarstad & Broch (1958) have all reported cases where patients developed angina chest pain during hypoxemia.

2) Performance of the hypoxemia test can result in sudden death (2/4 patients in one study) (Stewart & Carr, 1954).

3) Adverse reactions to the hypoxemia test include lightheadedness, sense of lack of air, moderate headaches, vasovagal attacks (syscope & nausea), drowsiness, fatigue, choking, heavy chest, tetany, fear and anxiety (Stewart & Carr, 1954). According to Levy *et al.*,

(1941), the dangers of the hypoxemia test are minimal, provided that the correct precautions are put in place and the test is only performed in certain groups of patients. Levy *et al.,* (1941) state that patients should not participate in the test if they have known congestive heart failure, have suffered a myocardial infarction in the past 4 months, have already undergone a provocation test that day, have had a positive stress test via an alternative method or have an abnormal resting ECG (Turner & Morton, 1952). These exclusion criteria limit the use of the hypoxemia test in patient groups where, on some occasions, the test would be most useful (Burchell *et al.,* 1948).

The hypoxemia test is no longer used in clinical practice because of the dangers and discomfort associated with performing the test. Up to 69% of patients have been shown to develop chest pain (Rothschild & Kissin, 1933) and even sudden death (2/4 patients) (Stewart & Carr, 1954) during the hypoxemia test.

In the present investigation, a milder form of inspired hypoxia (15% O_2) was used to induce less severe hypoxemia in conjunction with hypocapnia (20mmHg) induced by mechanical hyperventilation. The aim was to examine whether hypocapnia combined with 15% O_2 in inspired air would be sufficient enough to further reduce O_2 delivery to the myocardium and create a functional hypoxia within the heart which accentuates the small ECG changes seen during hypocapnia in 21% O_2 (increased T wave amplitude in leads V_{1-3}, chapter 4, page 98). It was not the aim of this investigation to simply repeat previous studies by reducing inspired O_2 to as low as possible in order to induce ECG changes. Instead, a milder form of inspired hypoxia that can be comfortably tolerated for extended periods of time was chosen which is equivalent to the O_2 inspired from air at an altitude of 6,700 feet (figure 5.1, Ward *et al.*,

Figure 5.1. Changes in minute ventilation (a) and heart rate (b) as inspired O_2 is reduced

a)

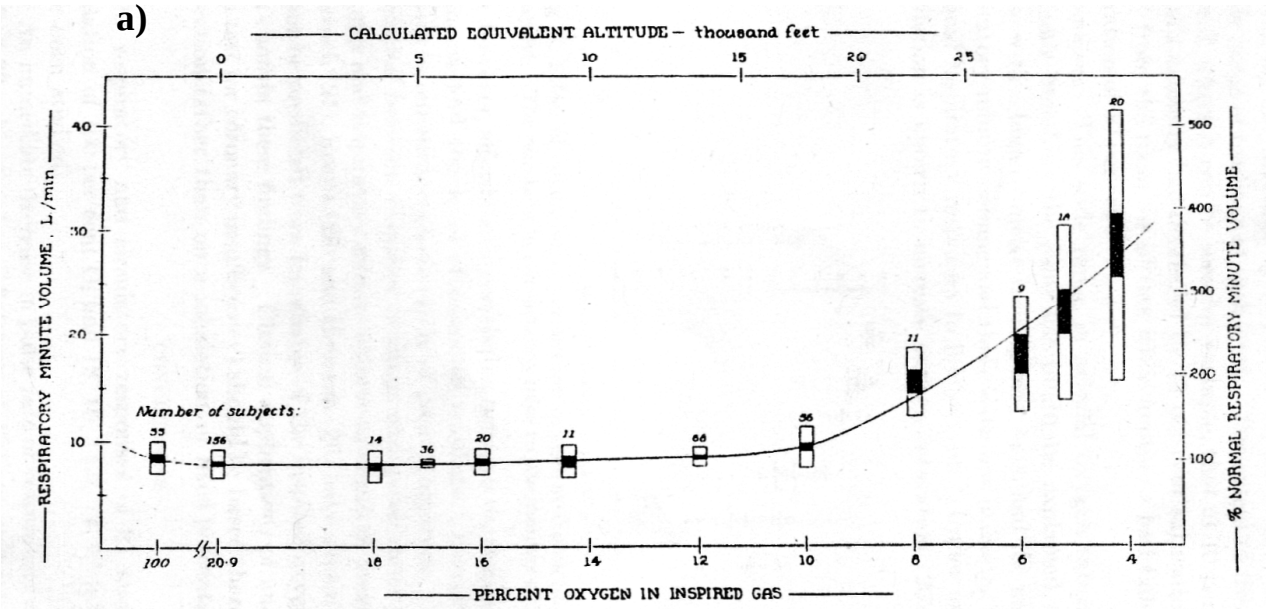


Figure 5.1a shows that noticeable changes in minute ventilation do not occur until inspired O_2 is reduced below 10% (equivalent to O_2 inspired at an altitude of 17,500 feet)

b)

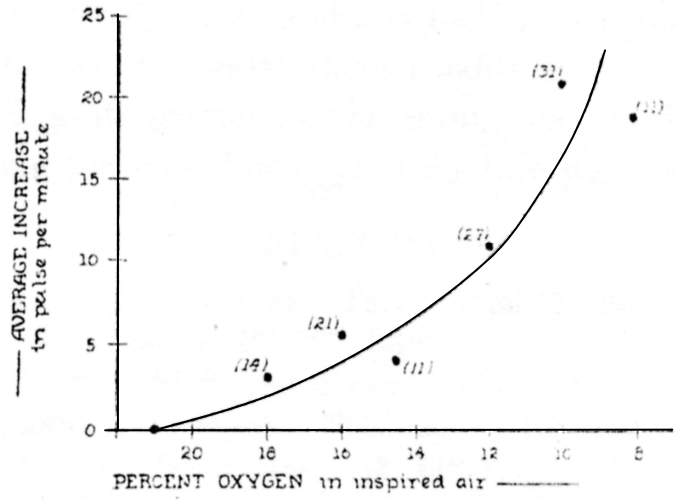


Figure 5.1b shows that changes in heart rate of >10bpm do not occur until inspired O_2 is reduced below 12% (equivalent to O_2 inspired at an altitude of 14,000 feet). Figures 5.1a & b from Dripps RD & Comroe JH (1947). The Effect of the Inhalation of High and Low Oxygen Concentrations on Respiration, Pulse Rate, Ballistocardiogram and Arterial Oxygen Saturation (Oximeter) of Normal Individuals. *American Journal of Physiology* 149, 277-291.

2000; Dripps & Comroe, 1947). 15% inspired O_2 is commonly tolerated by people taking routine commercial aircraft flights (Cottrell, 1988). The advantage of mechanically hyperventilating subjects in 15% O_2 instead of simply reducing their inspired O_2 is that hypocapnia stimulates a functional hypoxia within the heart (via coronary vasoconstriction and an increased affinity of haemoglobin for O_2) without substantially reducing arterial O_2 saturation which can result in distress and discomfort for the subject (Rothschild & Kissin, 1933; Stewart & Carr, 1954).

15% inspired O_2 does not stimulate carotid chemoreceptors sufficiently to cause large physiological changes in the body. Dripps & Comroe (1947) have shown that inspiration of 14.5% O_2 for 6-8 minutes causes trivial changes in instantaneous minute ventilation (V_E) (increase of 0.5 l/min) and heart rate (increase of 4 bpm) but significant changes do occur when inspired O_2 is reduced to 10% (increase in V_E of 1.3 l/min and heart rate of 21 bpm) (figure 5.1). To confirm these effects, measures of breathing frequency, tidal volume, V_E, instantaneous CO_2 production (VCO_2), heart rate and mean arterial blood pressure were taken during 10 minutes spontaneous inspiration of either 15% inspired O_2 or normal room air (normoxia) (5 minutes in each condition). Preliminary trials breathing 15% inspired O_2 revealed that 5 minutes is long enough to cause a drop and subsequent plateau in O_2 saturation at rest. This was therefore considered long enough to confirm whether 15% inspired O_2 is sufficient to cause changes in heart rate, blood pressure or ventilation.

5.3. Methods

Nine normal healthy subjects 23 ± 2 years old (aged 20-26 years old) (eight male) with no known cardiovascular disease were tested on two separate occasions. Subjects gave informed

consent and all experiments were approved by the Walsall Local Research Ethics Committee. All experiments were performed in accordance with the Declaration of Helsinki as stated by the American Physiological Society (2002). During each visit, subjects lay semi-recumbent on a bed and listened to the radio through headphones.

During each experimental trial, continuous blood pressure (from a finger plethysmograph, Finapres 2300), end-tidal CO_2 (from an in-line capnograph, Hewlett Packard 78354A) and O_2 saturation (from the finger using a pulse oximeter, N-200 series oximeter, Nellcor) were recorded.

5.3.1. Examining the effects of 15% inspired O_2 on spontaneous breathing

The effects of inspiration of 15% O_2 on spontaneous minute ventilation and metabolic rate were assessed with the breathing apparatus previously described (chapter 2.6, page 32). A 3 lead ECG (for determination of heart rate only) and mean arterial blood pressure were continuously recorded for a period of 10 minutes during which subjects inhaled room air for 5 minutes (*normoxia*) and 15% O_2 in N_2 for 5 minutes (*hypoxia*). The order in which room air and 15% O_2 were inspired was established at random. A certified gas mixture of 15% O_2 in 85% N_2 was used for all experiments in hypoxia. ECG, $PetCO_2$, O_2 saturation and breathing data were recorded by Spike2 data acquisition software via a CED power 1401 PC interface. Breathing frequency, tidal volume, V_E, VCO_2 and V_E/VCO_2 ratio were derived from the breathing apparatus data as previously described (chapter 2.6.1, page 34).

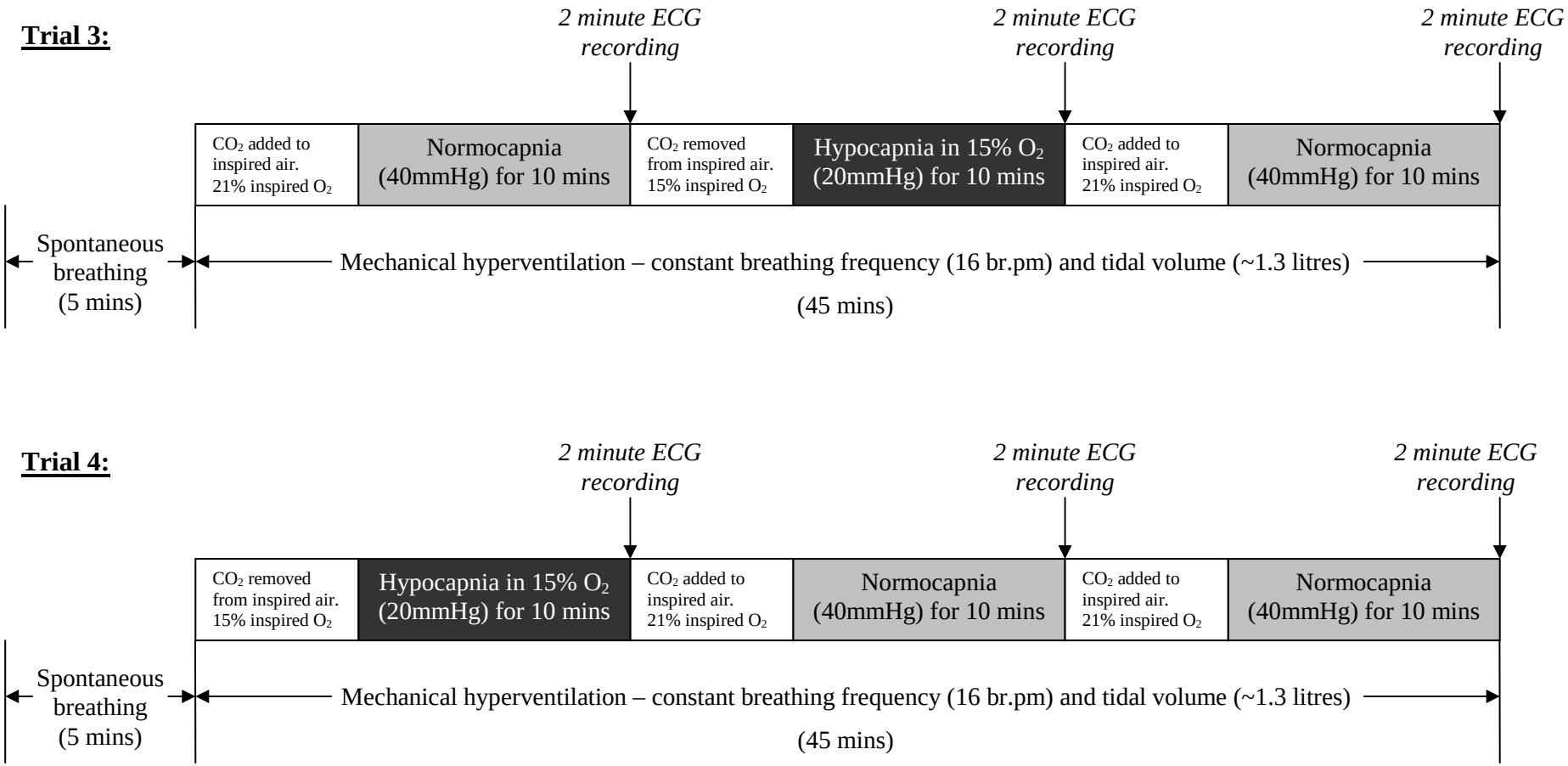
All data are expressed as means ± standard error. Statistical analysis was performed on the breathing data by sampling all data points once every 5s (the minimum realistic resolution of

instantaneous breathing measurements). Analysis was performed by repeated measures analysis with two within subject factors (normoxia vs. hypoxia [condition] and effects over time [time]). Where the repeated measures analysis was significant according to the Huynh-Feldt method, simple contrasts analysis was performed to compare subsequent time points against the first initial time point (zero). Significant *F* values were found for O_2 saturation (time, $F = 9.2$, $P < 0.001$, condition, $F = 68.5$, $P < 0.01$, condition*time, $F = 21.7$, $P < 0.001$). Secondary analysis was performed to search for any consistent pattern of significant contrasts in the condition*time interactions. There were no significant *F* values for frequency, minute ventilation, VCO_2, Ve/VCO_2, heart rate and blood pressure. There were significant *F* values for tidal volume (condition only, $F = 9.5$, $P < 0.05$), but since the effects of time and time*condition were not significant, these were not pursued any further.

5.3.2. Examining the effects of hypocapnia in 15% inspired O_2

Hypocapnia in 15% inspired O_2 (*hypocapnic hypoxia*) was induced by removing the additional CO_2 from inspired air (causing $PetCO_2$ to fall) and replacing it with a 15% O_2 in N_2 gas mixture from a pressurised cylinder. Mechanical hyperventilation in normocapnia, hypocapnia and hypocapnic hypoxia were induced using a mechanical ventilator (Däger Evita II) as previously described (chapter 2.3, page 26). Subjects were mechanically hyperventilated in four trials; twice in normocapnia and hypocapnia ($PetCO_2$ of 20mmHg) (figure 4.1, page 107) and twice in normocapnia and hypocapnic hypoxia (figure 5.2). Breathing frequency and tidal volume were kept constant throughout all stages of mechanical hyperventilation.

Figure 5.2. Order and duration that normocapnia and hypocapnic hypoxia were induced during each trial in mechanical hyperventilation (trials 1 & 2 are shown in figure 4.1)



All 12 leads of the ECG were recorded during mechanical hyperventilation with 10 Blue sensor ECG electrodes (P-00-S/50, Ambu) which were placed in the standard electrode placement defined by Kligfield *et al.*, (2007) (figure 2.4, page 43). All ECG leads were recorded simultaneously, as previously described (chapter 2.9.2, page 50). ECG waveforms were averaged for each lead over two minute periods in each condition (as previously described in chapter 2.9.3, page 55). R and T wave amplitudes, ST segment height and QT interval duration were measured from these averaged waveforms. Mean wave amplitudes and durations were calculated for each condition, in all subjects, across all trials. Data from each condition are presented with non-gender specific clinical thresholds for normality (Wagner, 2008; 2009; Rautaharju *et al.*, 2009). ST segment height and T wave amplitude (recorded in the transverse plane) were also separated by gender and compared to gender specific clinical thresholds as recommend by Wagner *et al.*, (2009) and Rautaharju *et al.*, (2009).

The statistical significance between R and T wave amplitudes, ST segment height, QTc interval duration, heart rate and mean arterial pressure during mechanical hyperventilation in normocapnia, hypocapnia and hypocapnic hypoxia was investigated. Statistical analysis was performed using repeated measures analysis with application of the Huynh-Feldt correction for nonsphericity. Separate analysis was performed in each lead as it was assumed that all 12 leads are independent and uncorrelated. No significant F values were found for condition in any wave amplitudes or durations in all leads. When data were separated by gender, significant F values were found between T wave amplitudes in male subjects in lead V_2 (F = 5.0, $P < 0.05$). The sources of this significance were investigated using a 2-tail paired t-test.

The statistical significance between O_2 saturations measured in normocapnia, hypocapnic hypoxia (during mechanical hyperventilation) and hypoxia (during spontaneous breathing) was also investigated. Statistical analysis was performed using repeated measures analysis with application of the Huynh-Feldt correction for nonsphericity. Significant *F* values were found for comparisons of O_2 saturation in normocapnia, hypocapnic hypoxia and hypoxia ($F = 97.4$, $P < 0.001$). The source this of significance was investigated using a 2-tail paired t-test.

5.4. Results

5.4.1. Normoxia vs. hypoxia

As expected, spontaneous inspiration of 15% O_2 caused a significant reduction in O_2 saturation from 55 seconds onwards (figure 5.3). After 5 minutes, O_2 saturation was reduced by 4 ± 0% to 93 ± 1%. This hypoxia did not cause significant changes in heart rate or mean arterial pressure (figure 5.4).

Figure 5.3. Spontaneous inspiration of 15% Oxygen (hypoxia) causes significant decreases in O_2 saturation in 9 normal subjects

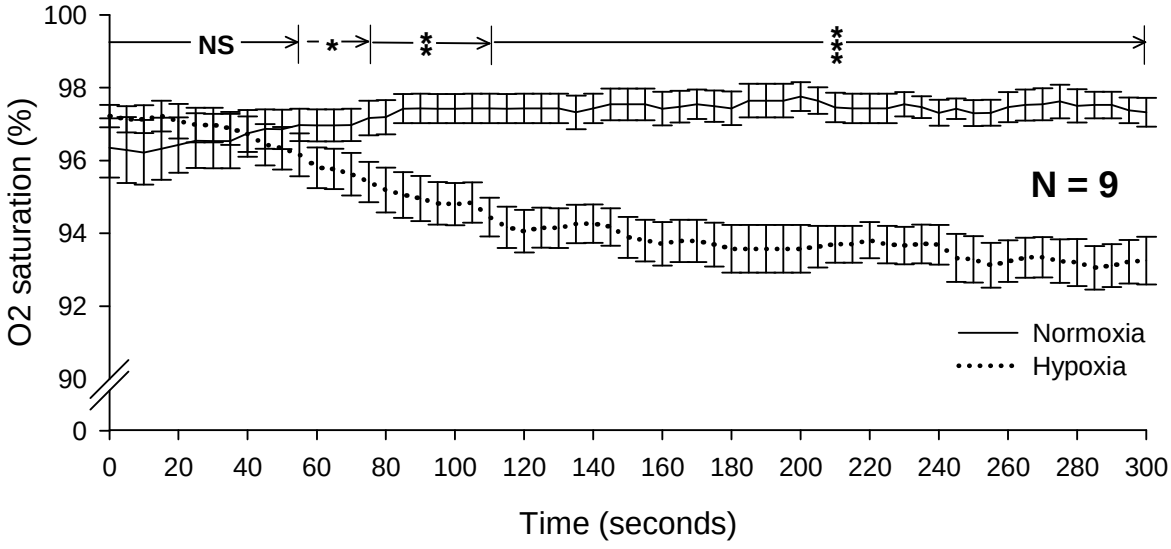


Figure 5.3. Mean ± SE O_2 saturation during 5 minutes of normoxia and 5 minutes of hypoxia in 9 normal subjects. * $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$, normoxia vs. hypoxia over time.

Figure 5.5 shows that there was no significant difference in breathing frequency, tidal volume or instantaneous V_E during 15% inspired O_2 compared to normoxia. As expected, instantaneous VCO_2 (indicating metabolic rate) was unaffected by inspiration of 15% O_2 (figure 5.6). Therefore, instantaneous V_E/VCO_2 ratio remained unchanged throughout the 10 minutes of spontaneous breathing of different gas mixtures indicating that the subjects were not hyper/hypo-ventilating at any point during the experiment (figure 5.6).

Figure 5.4. 5 minutes of spontaneous inspiration of 15% Oxygen (hypoxia) does not cause significant changes in heart rate or mean arterial pressure in 9 normal subjects.

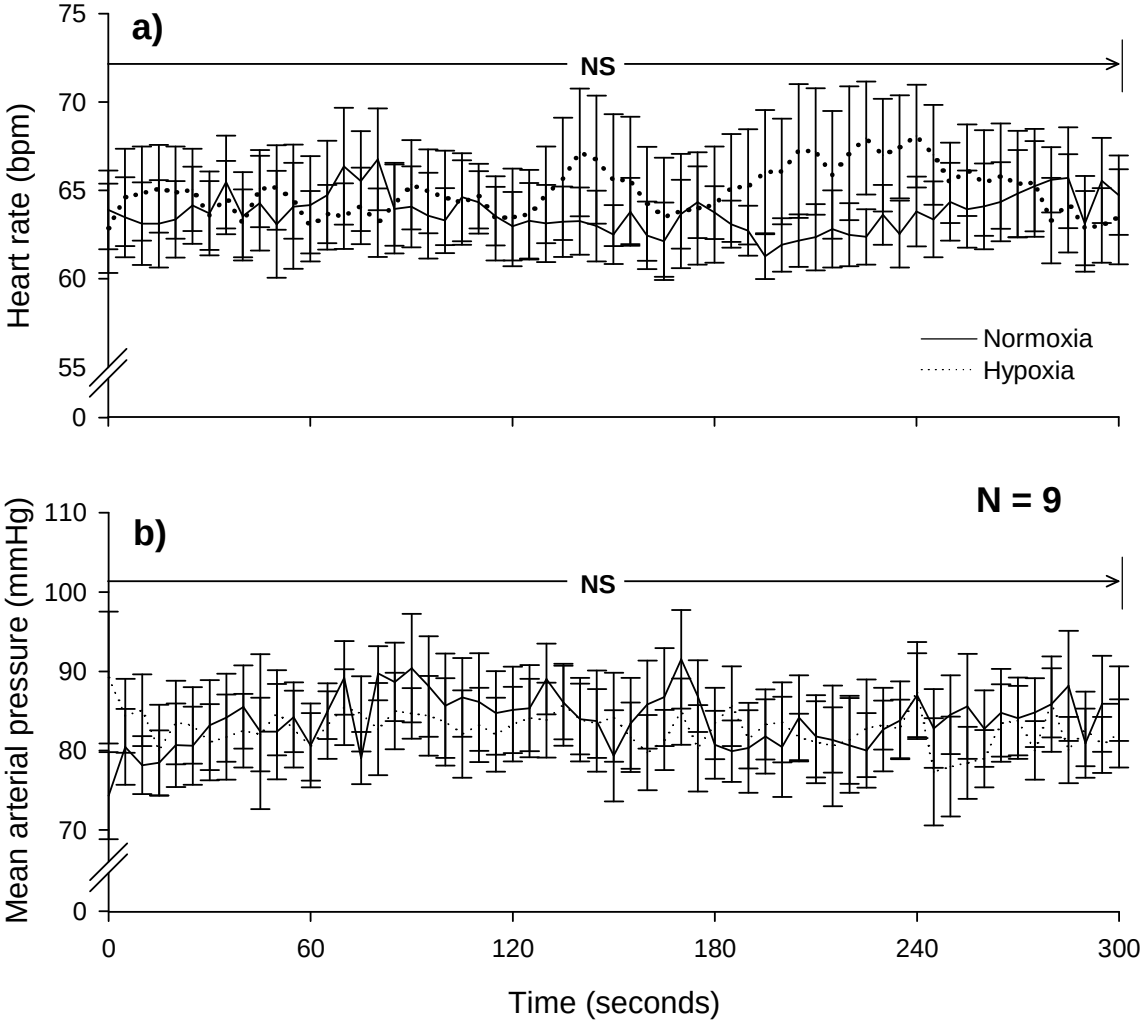


Figure 5.4. Mean ± SE Heart rate (a) and mean arterial pressure (b) during 5 minutes of normoxia and 5 minutes of hypoxia in 9 normal subjects. All data points in hypoxia, *NS* vs. normoxia over time.

Figure 5.5. As expected, spontaneous inspiration of 15% Oxygen (hypoxia) does not cause a significant change in breathing frequency, tidal volume or minute ventilation in 9 normal subjects

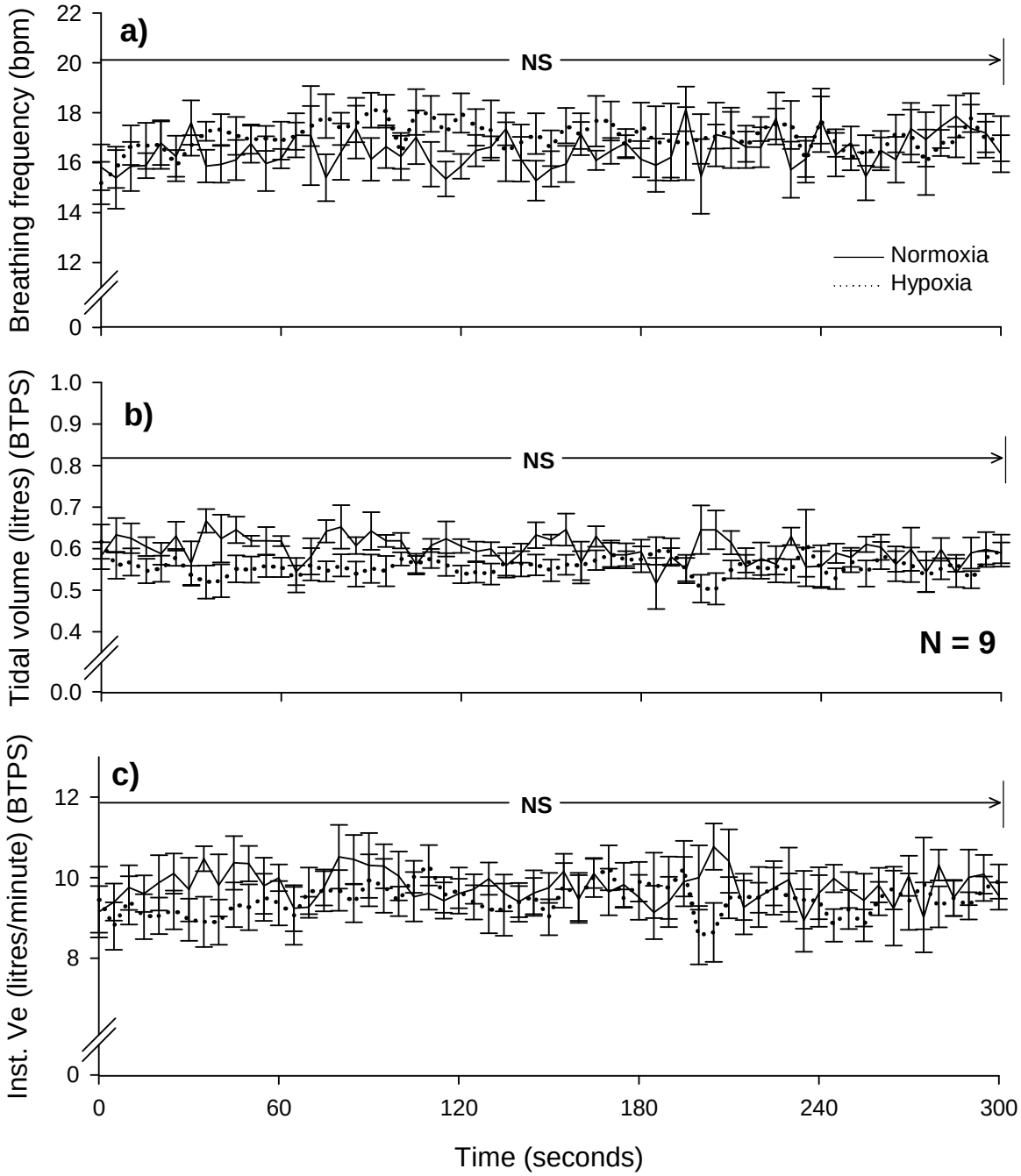


Figure 5.5. Mean ± SE Breathing frequency (a), tidal volume (b) and instantaneous minute ventilation (Inst. Ve) (c) during 5 minutes of normoxia and 5 minutes of hypoxia in 9 normal subjects. All data points in hypoxia, *NS* vs. normoxia.

Figure 5.6. As expected, spontaneous inspiration of 15% Oxygen (hypoxia) does not cause significant changes in metabolic rate, expressed as instantaneous VCO_2, and does not cause hyper/hypo-ventilation (Ve/VCO_2 ratio does not change) in 9 normal subjects.

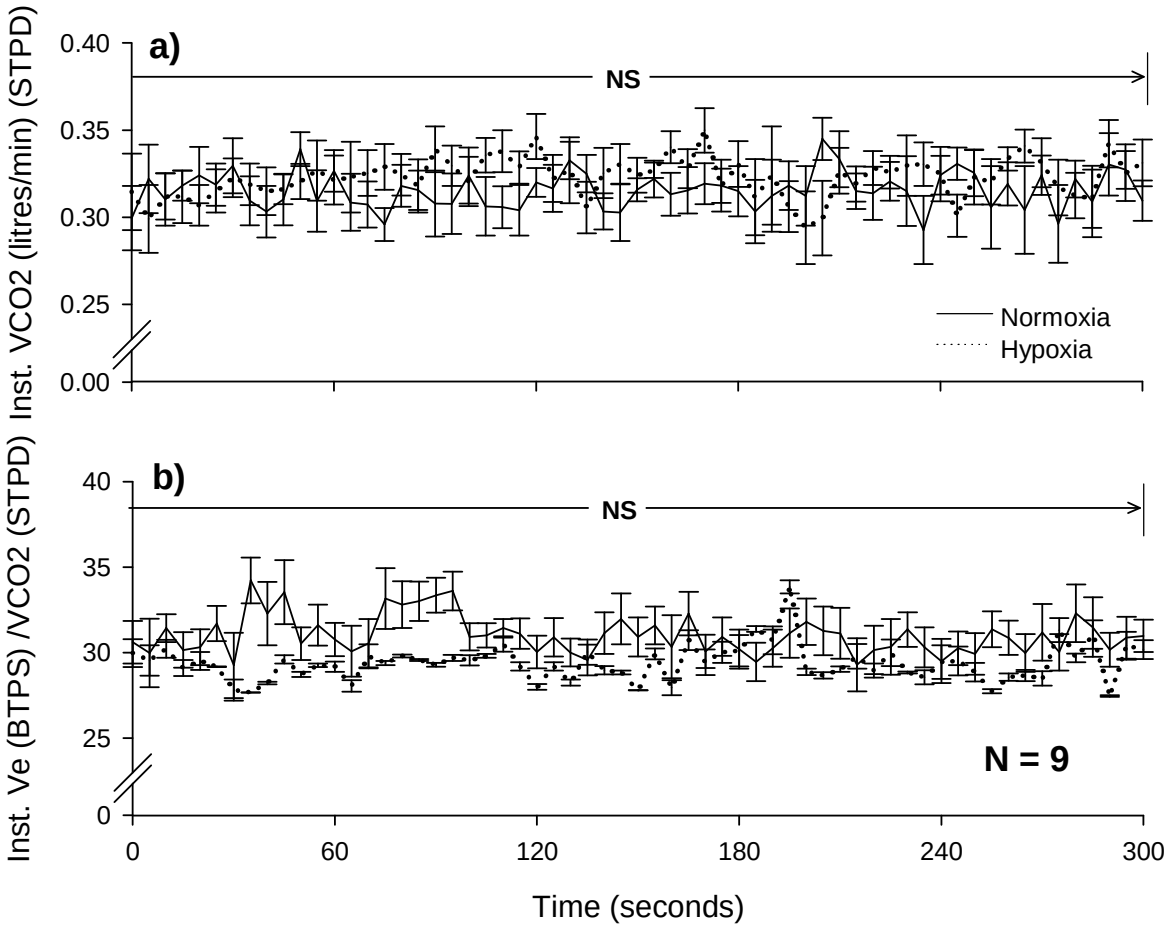


Figure 5.6. Mean ± SE Metabolic rate expressed as intantaneous CO_2 production (Inst. VCO_2) (a) and instantaneous Ve/VCO_2 ratio (b) (showing hyper/hypo-ventilation) during 5 minutes of normoxia and 5 minutes of hypoxia in 9 normal subjects. All data points in hypoxia, *NS* vs. normoxia.

5.4.2. Heart rate, blood pressure and ECG changes during hypocapnic hypoxia

Mechanical hyperventilation caused a decrease in $PetCO_2$ of 19 ± 0mmHg during hypocapnia and hypocapnic hypoxia from baseline (normocapnia) in all nine subjects (figure 5.7). Hypocapnic hypoxia did not cause the significant change in O_2 saturation seen in hypoxia in spontaneous breathing (99% in normocapnia [$P < 0.001$ vs. hypoxia] vs. 93% in hypoxia vs. 99% during hypocapnic hypoxia [$P < 0.001$ vs. hypoxia]) (figure 5.8). Both hypocapnia and hypocapnic hypoxia had no significant effect on heart rate (57 ± 1 bpm in normocapnia vs. 59

± 2 bpm in hypocapnia vs. 65 ± 3 bpm in hypocapnic hypoxia [$P > 0.05$]) or mean arterial pressure compared to normocapnia (93 ± 4mmHg in normocapnia vs. 86 ± 6mmHg in hypocapnia vs. 93 ± 4mmHg in hypocapnic hypoxia [$P > 0.45$]) (figure 5.9).

Figure 5.7. Mechanical hyperventilation caused $PetCO_2$ to decrease by 19mmHg during hypocapnia and hypocapnic hypoxic conditions

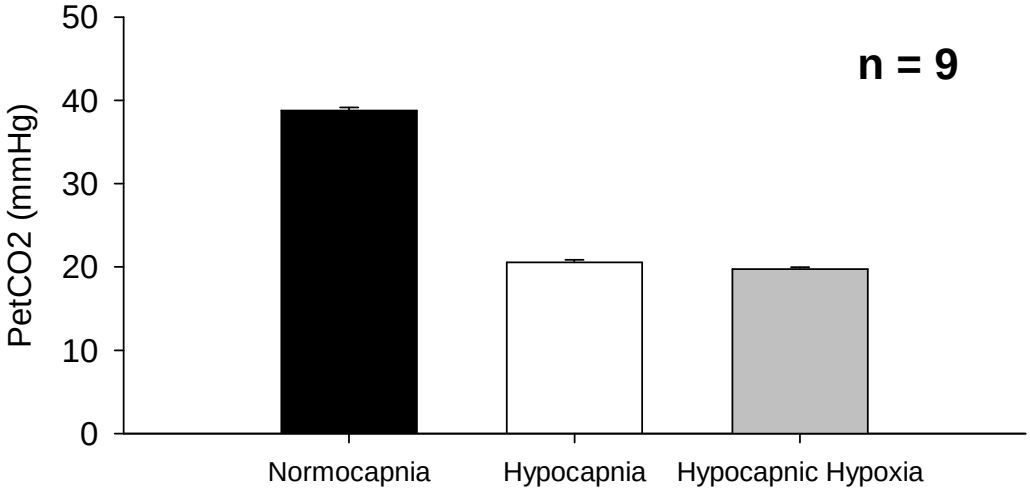


Figure 5.7. Mean ± SE $PetCO_2$ during mechanical hyperventilation in normocapnia, hypocapnia and hypocapnic hypoxia in 9 subjects.

Figure 5.8. The decrease in O_2 saturation during hypoxia at rest does not occur during hypocapnic hypoxia in mechanical hyperventilation

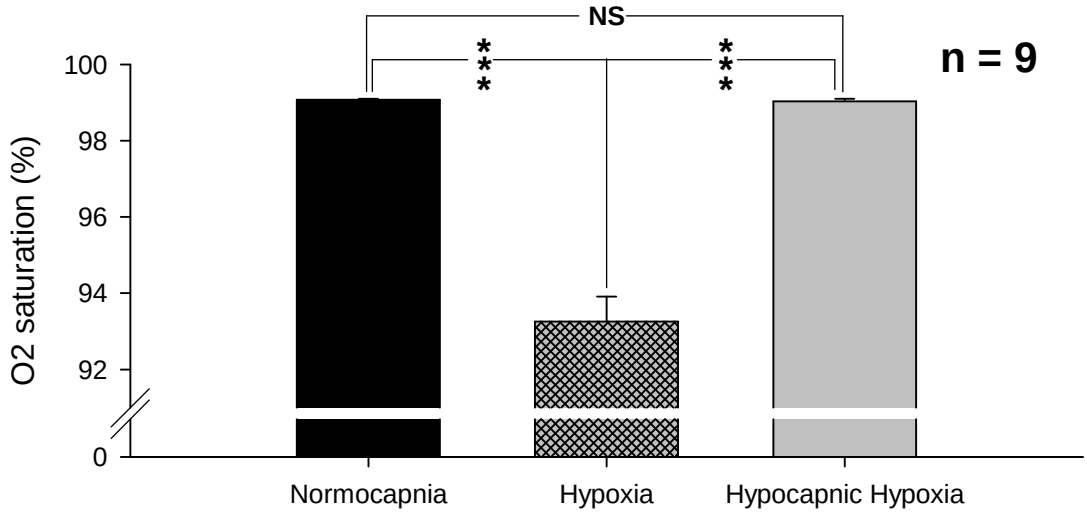


Figure 5.8. Mean ± SE O_2 saturation of arterial blood during mechanical hyperventilation in normocapnia and hypocapnic hypoxia and spontaneous breathing in hypoxia. % saturation in hypoxia, ***$P < 0.001$ vs. normocapnia or hypocapnic hypoxia, normocapnia, *NS* vs. hypocapnic hypoxia.

Figure 5.9. The addition of hypoxia to hypocapnia does not cause significant changes in heart rate or blood pressure in 9 subjects.

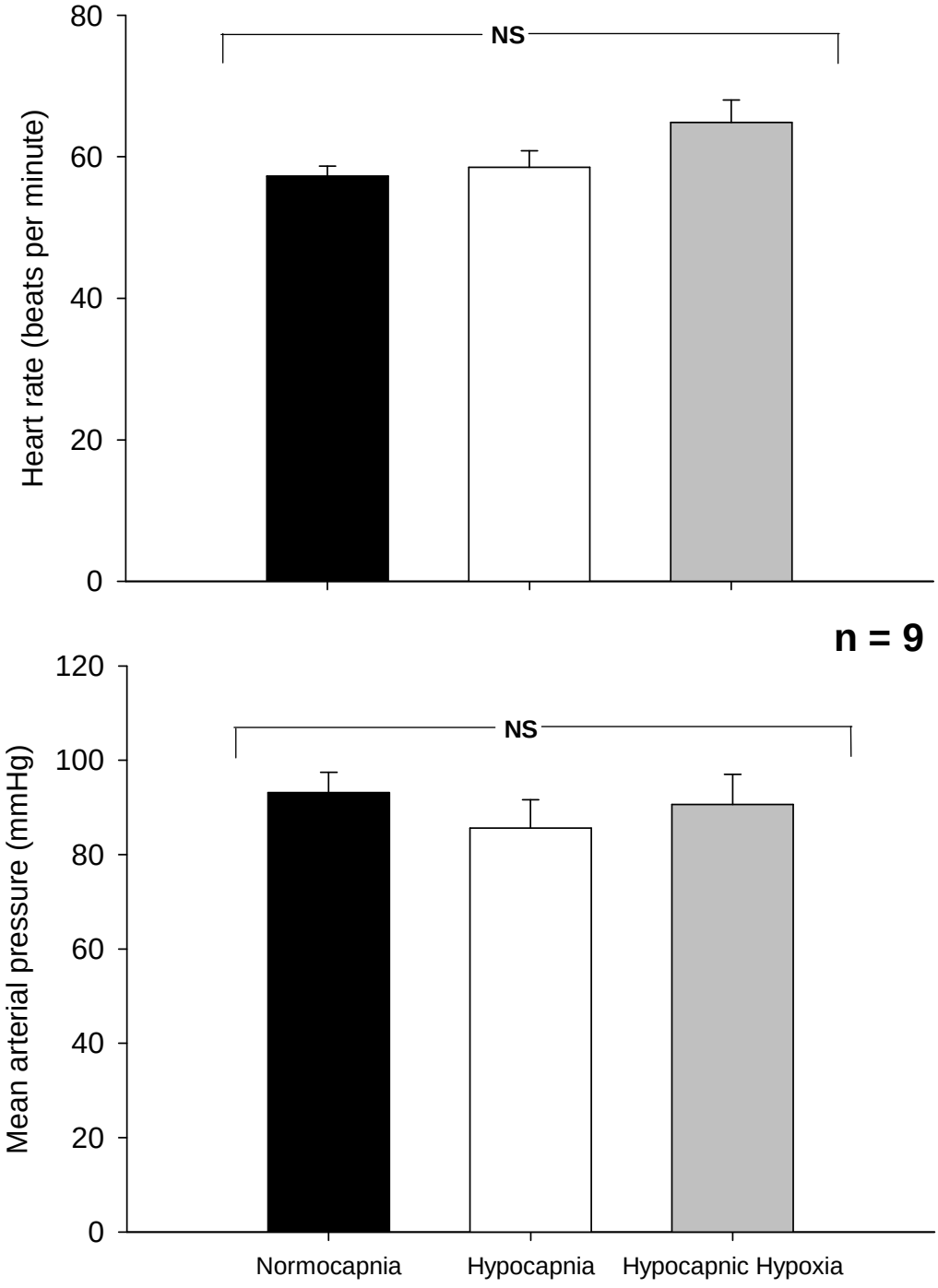


Figure 5.9. Mean ± SE heart rate and blood pressure changes during mechanical hyperventilation in normocapnia, hypocapnia and hypocapnic hypoxia in 9 subjects. Data points in all conditions, *NS* vs. normocapnia.

Hypocapnic hypoxia did not augment any of the small ECG changes seen during hypocapnia in 21% O_2. Figure 5.10 shows that in nine subjects, T wave amplitude increased during hypocapnia in the anteroseptal leads (V_{1-3}) but this was not statistically significant (increase of 0.05 ± 0.02mV [>38%]) in V_1, 0.09 ± 0.03mV [>13%] in V_2 and 0.08 ± 0.03mV [>11%] in V_3 [$P > 0.65$]) (figure 5.10). Hypocapnic hypoxia did not cause significant increases in T wave amplitude in any leads, nor did it significantly augment T wave changes originally seen in the anteroseptal leads (V_{1-3}) during hypocapnia in 21% O_2 (chapter 4, page 107) (figure 5.10). As expected, no R wave changes occurred in any leads (figure 5.11). All T wave amplitudes measured during hypocapnic hypoxia were within clinical thresholds (>0.5-1.0mV) for normality.

Figure 5.12 shows that no statistically or clinically significant changes in ST segment height occurred during hypocapnia or hypocapnic hypoxia in any of the 12 leads of the ECG. When T wave and ST segment data were separated by gender in the transverse plane, statistically significant increases in T wave amplitude appeared in lead V_2 in males during hypocapnia (0.13 ± 0.02mV [18%] vs. normocapnia [$P < 0.05$]) and hypocapnic hypoxia (0.09 ± 0.03mV [13%] vs. normocapnia [$P < 0.05$]) (figure 5.13a). No clinically significant changes occurred in either the male or female subjects (figure 5.13). No other gender differences occurred in T wave amplitude or ST segment height during mechanical hyperventilation (figure 5.13 and 5.14).

Hypocapnic hypoxia did not cause prolongation of the QTc interval in any of the 12 leads of the ECG (figure 5.15).

Figure 5.10. The addition of hypoxia to hypocapnia does not significantly alter T wave amplitude in any of the 12 leads of the ECG.

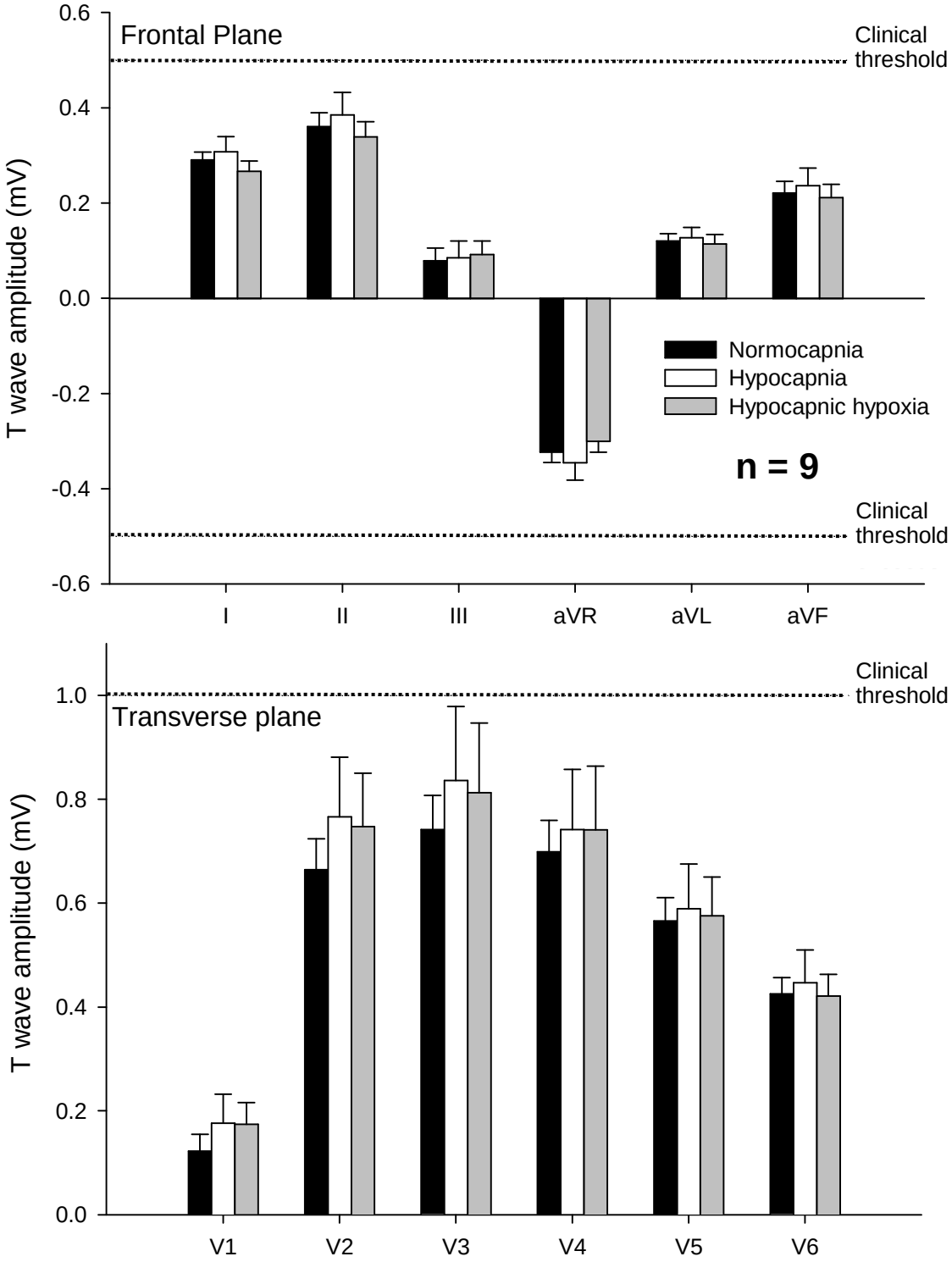


Figure 5.10. Mean ± SE T wave amplitudes during mechanical hyperventilation in normocapnia, hypocapnia and hypocapnic hypoxia. Data points in all conditions, *NS* vs. normocapnia. Dotted lines indicate upper boundries for normal T wave amplitudes

Figure 5.11. The addition of hypoxia to hypocapnia does not have any significant affect on R wave amplitude in any of the 12 leads of the ECG in 9 normal subjects

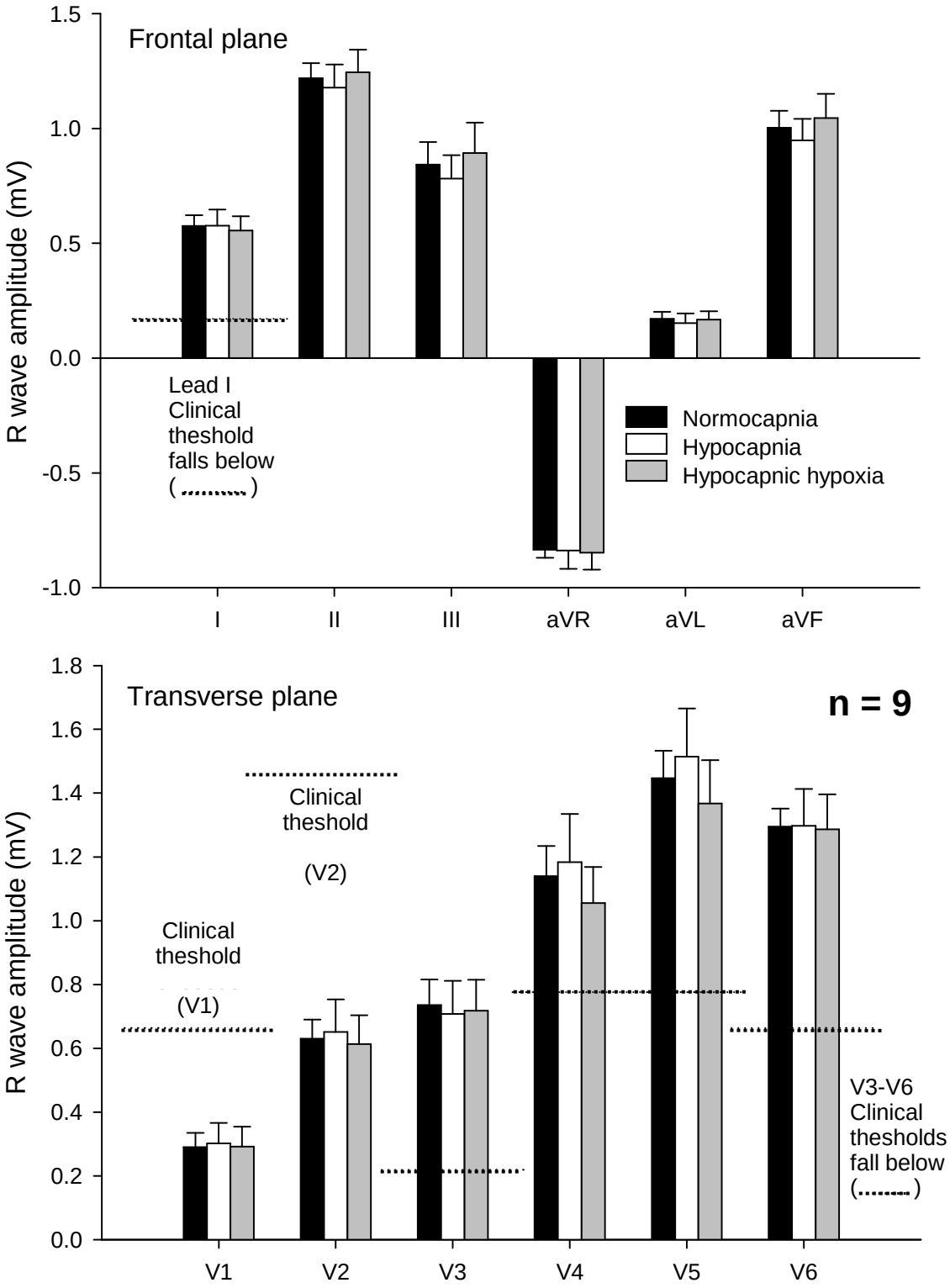


Figure 5.11. Mean ± SE R wave amplitudes during mechanical hyperventilation in normocapnia, hypocapnia and hypocapnic hypoxia. Data points in all conditions, *NS* vs. normocapnia. Dotted lines indicate upper boundries for normal T wave amplitudes

Figure 5.12. The addition of hypoxia to hypocapnia did not cause significant ST segment changes in any of the 12 leads of the ECG.

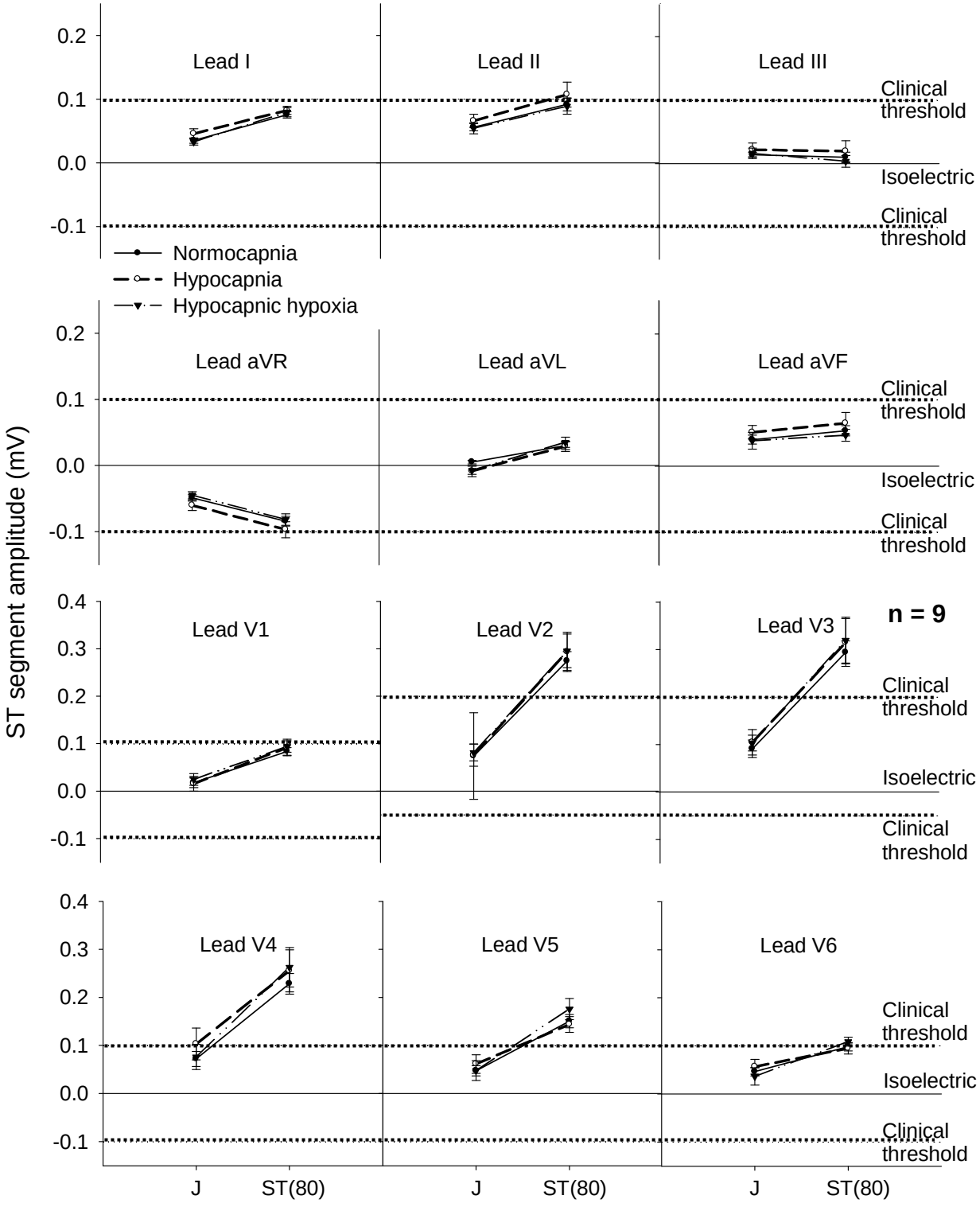


Figure 5.12. Mean ± SE ST segment height are defined by deviation of the J point from the isoelectric line. The extent of these changes is estimated by deviation of the ST(80) point. All points in all conditions, *NS* vs. normocapnia. Dotted line represents clinically significant deviation from isoelectric line.

Figure 5.13. The effects of hypocapnia & hypocapnic hypoxia on T wave amplitude in the transverse plane are not altered when data is seperated by gender except in lead V_2.

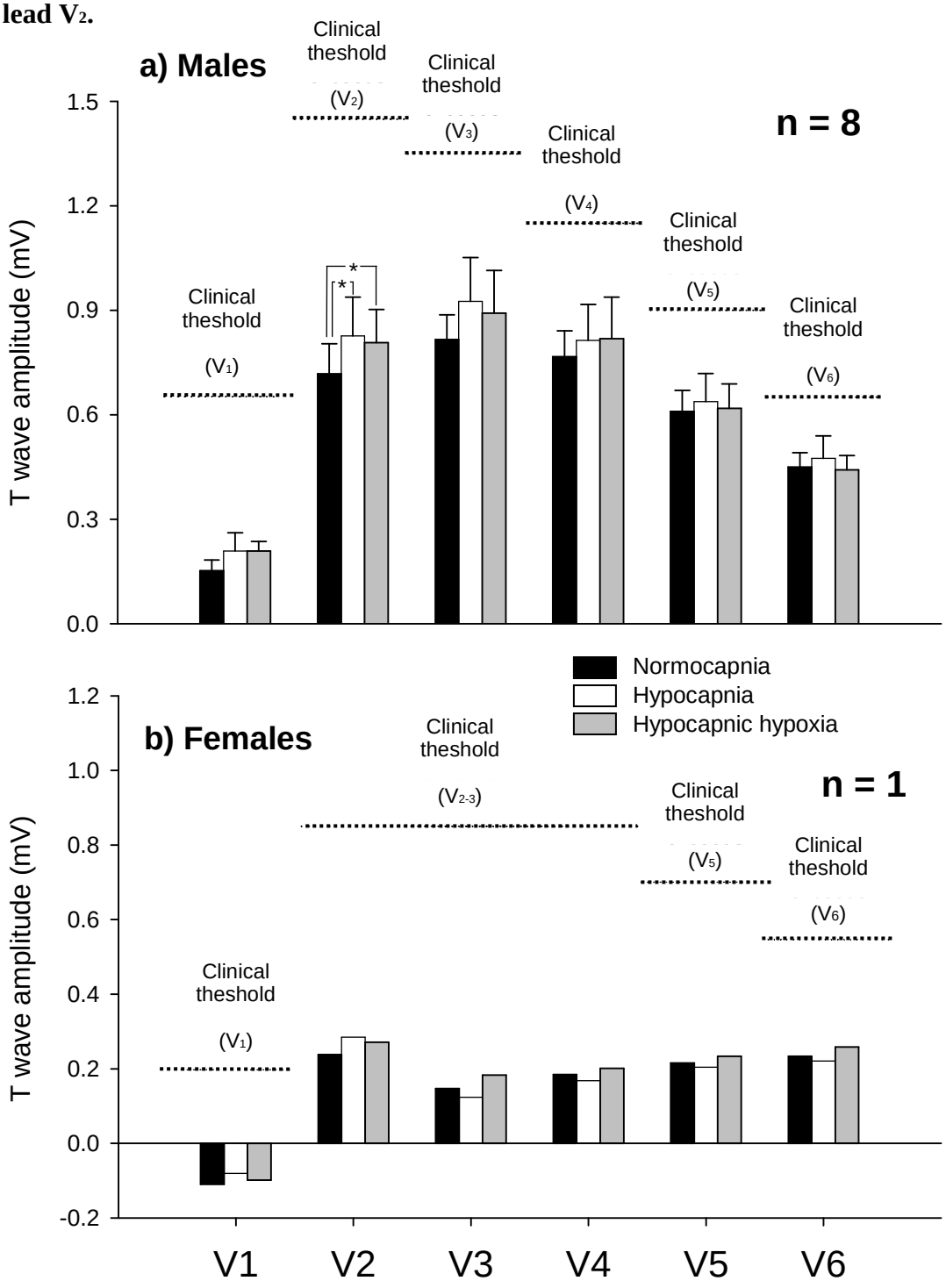


Figure 5.13. Mean ± SE T wave amplitude in the transverse plane during normocapnia, hypocapnia and hypocapnic hypoxia in males (a) and 1 female (b). Gender specific clinical thresholds for abnormality (.....) in the T wave are shown. In males, hypcapnia & hypcapnic hypoxia, *$P < 0.05$ vs. normocapnia. All other T wave amplitudes in all conditions are *NS* vs. each other.

Figure 5.14. The effects of hypocapnia & hypocapnic hypoxia on the ST segment height in the transverse plane are not altered when data is controlled for gender.

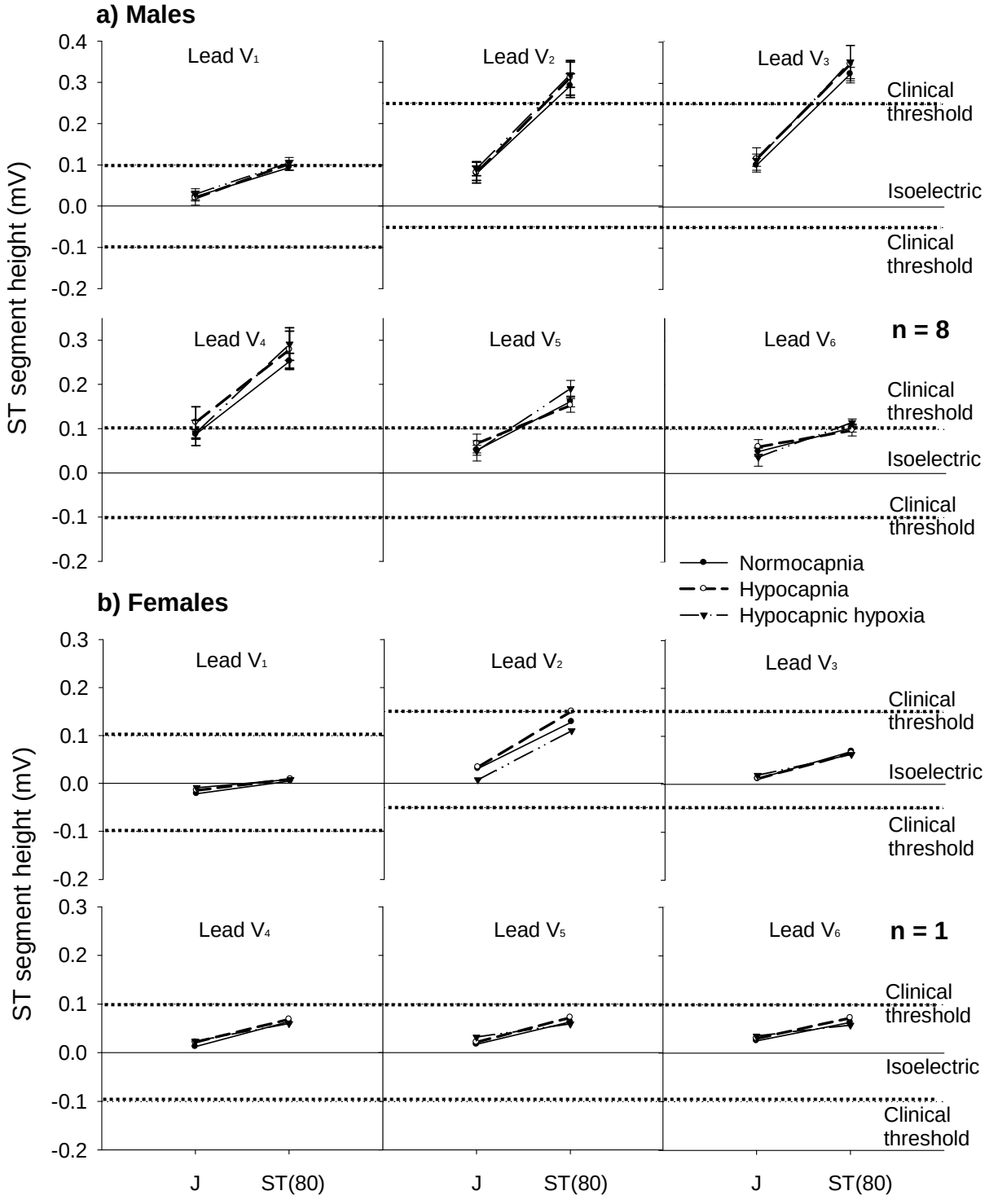


Figure 5.14. Mean ± SE of ST segment height (J point & ST[80] point) in the transverse plane in males (a) and 1 female (b). Dotted lines are gender sepecific and represent clinically significant deviation from isoelectric line (straight line). All ST heights in all conditions, *NS* vs. each other.

Figure 5.15. The addition of hypoxia to hypocapnia does not cause significant QTc interval prolongation in any of the 12 leads of the ECG of 9 normal subjects

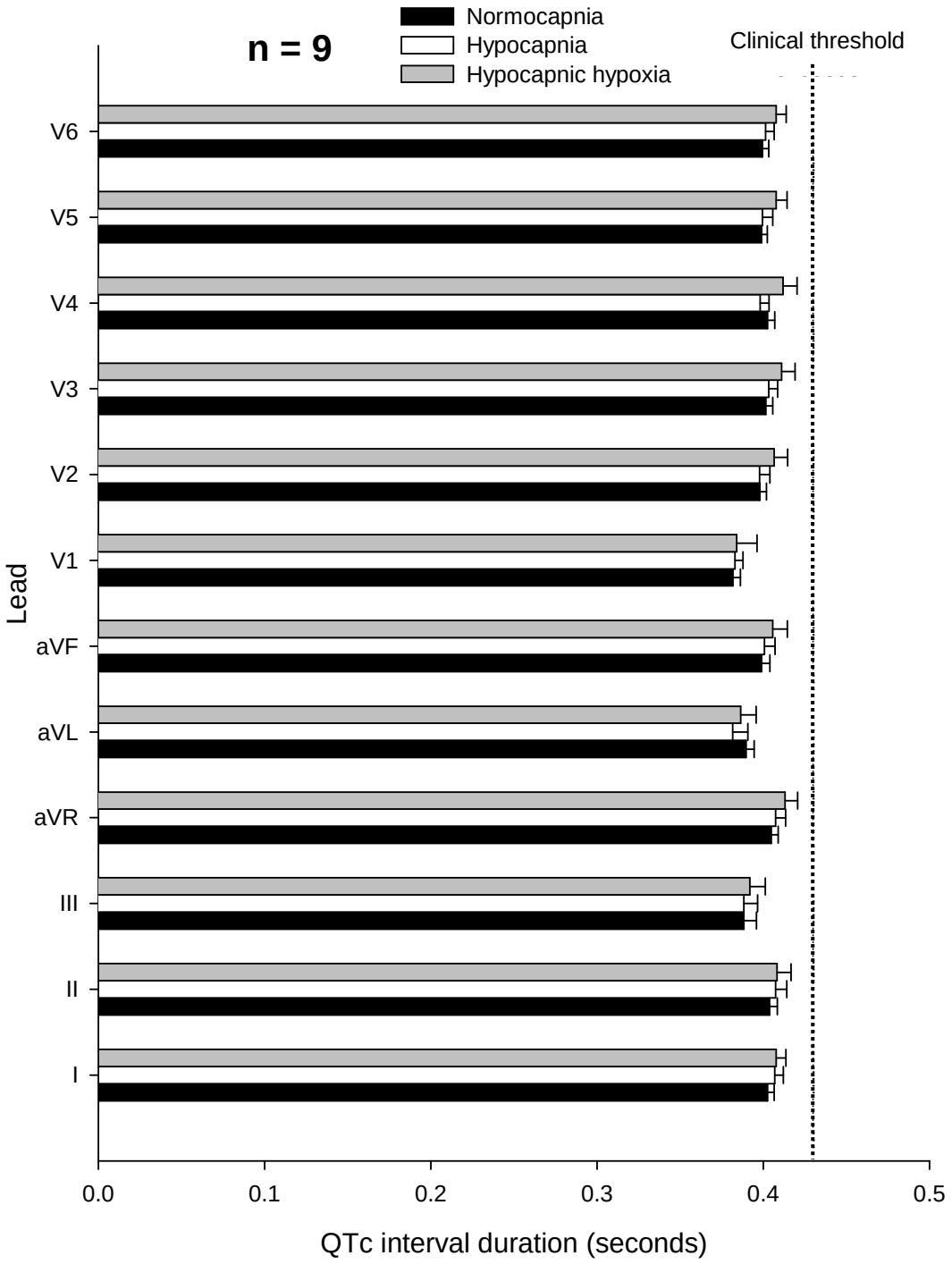


Figure 5.15. Mean ± SE QTc interval duration during mechanical hyperventilation in normocapnia, hypocapnia and hypocapnic hypoxia. All durations in all conditions, *NS* vs. normocapnia. The dotted line indicates the upper boundry for non-ischemic QTc interval duration.

5.5. Discussion

Reducing inspired O_2 to 15% during hypocapnia, induced by mechanical hyperventilation, does not augment the small ECG changes seen during hypocapnia in 21% O_2 in normal healthy subjects.

5.5.1. Absence of physiological changes during hypoxia

As expected, inspiration of 15% O_2 caused O_2 saturation to decrease (by up to 4.2 ± 0.4%) in all subjects. If inspired O_2 had been reduced to 12-7.5%, a larger decrease in O_2 saturation (of 12-33%) would be expected (Broch, 1972a; Broch, 1972b; Levy *et al.*, 1938; Barach *et al.*, 1941). The aim of the present investigation was not to continually reduce inspired O_2 until chest pain and discomfort appear. Therefore, 15% inspired O_2 was used because it is equivalent to an altitude of 6,700 ft and is known to be well tolerated by people taking routine commercial aircraft flights (Cottrell, 1988; Ward *et al.*, 2000).

This study attempted to confirm that spontaneous inspiration of 15% O_2 only causes trivial changes in heart rate or mean arterial pressure (figure 5.4). Dripps & Comroe (1947) showed that breathing a 14.5% O_2 mixture for 6-8mins causes a small but significant increase in heart rate of 4 bpm in resting subjects. This increase in heart rate was thought to be caused by carotid chemorecepter stimulation caused by reduced O_2 availability in arterial blood. In the present investigation, no consistent changes in heart rate or blood pressure were seen during spontaneous inspiration of 15% O_2.

Analysis of ventilation during normoxia and hypoxia revealed that breathing frequency, tidal volume and instantaneous V_E were not significantly affected by hypoxia (figure 5.5). These

findings confirm those of Dripps & Comroe (1947) who reported that ventilation is not affected by inspiration of 14.5% O_2. The absence of changes in V_E and VCO_2 mean that the V_E/VCO2 ratio was unaffected by hypoxia (figure 5.6). It can therefore be concluded that subjects did not hypo/hyper-ventilate during the spontaneous inspiration of 15% O_2.

5.5.2. Physiological changes during hypocapnic hypoxia

During mechanical hyperventilation in nine subjects, hypocapnia and hypocapnic hypoxia caused no significant change in heart rate (1 ± 1 bpm in hypocapnia and 8 ± 3 bpm in hypocapnic hypoxia). Previous studies suggest that an increase in heart rate might have been expected during hypocapnic hypoxia, even though the inspired O_2 was only reduced to 15% (Dripps & Comroe, 1947). However, the lack of decrease in O_2 saturation suggests that the stimulus for an increase in heart rate (decrease in O_2 availability in arterial blood [Dripps & Comroe, 1947; Rutherford *et al.*, 2005]) may have been removed.

Mechanical hyperventilation in hypocapnia and 15% O_2 did not accentuate the increases in T wave amplitude seen previously in the anteroseptal leads (V_{1-3}) (chapter 4.4.1, page 112), nor did it cause significant T wave changes in any other leads. Again, hypocapnia did not cause statistically or clinically significant changes in ST height. The fact that inspiration of 15% O_2 did not accentuate the increases in T wave amplitude seen during hypocapnia in 21% O_2, suggests that these original T wave changes were not caused by a functional hypoxia within the myocardium.

When data from male subjects and 1 female subject were separated, statistically significant differences in the T wave amplitude of lead V_2 in males were revealed during hypocapnia and

hypocapnic hypoxia. In addition, clinically (but not statistically) significant ST segment elevation was seen in V_4 during hypocapnia. Hypocapnic hypoxia did not significantly augment T wave amplitude or ST segment height from that seen during hypocapnia in 21% O_2. This suggests that hypocapnia in 15% inspired O_2 does not significantly alter ECG changes seen during hypocapnia alone.

The significant decrease in O_2 saturation seen during spontaneous breathing in 15% O_2 was not seen in the presence of hypocapnia during mechanical hyperventilation. The reduction in the partial pressure of CO_2 and the increase in blood pH during hypocapnia causes the affinity of haemoglobin for O_2 to augment (Roughton, 1964). In addition, according to the alveolar gas equation (Fenn *et al.*, 1946), as the partial pressure of alveolar CO_2 ($PACO_2$) decreases, the partial pressure of alveolar O_2 (PAO_2) increases. Therefore, during hypocapnia, the maintenance of normal O_2 saturations could be explained by increased PAO_2, increased uptake of O_2 at the lungs and decreased dissociation of O_2 into the working tissues.

This represents a potential limitation to this study which might explain the lack of observed ECG changes. It appears that during mechanical hyperventilation, the augmented breathing frequency and tidal volume increased the amount of O_2 being exchanged in the lung, therefore compensating for the lower proportion of O_2 in the air. The absence of any changes in O_2 saturation suggests that O_2 delivery to the myocardium during hypocapnic hypoxia was no different to O_2 delivery during hypocapnia in normal room air. To achieve the desired reduction in O_2 saturation, it would therefore be necessary to reduce inspired O_2 further, to a level which would not normally be considered safe in normal resting conditions.

5.5.3. Conclusions

The findings of this study suggest that inspiration of 15% O_2 during mechanical hyperventilation in hypocapnia was not sufficient to reduce O_2 availability further and cause a functional hypoxia within the myocardium of normal subjects.

The lack of ECG changes occurred because a functional hypoxia within the myocardium could not be induced in healthy subjects. It appears that inspiring 15% O_2 during mechanical hyperventilation does not have any additional effect on PaO_2, coronary blood flow or O_2 availability in the myocardium.

It is known that compensatory vasodilatation occurs in the coronary arteries during significant hypoxia which causes blood flow to increase (Kaufmann *et al.*, 2001; Arbab-Zadeh *et al.*, 2009). However, this is unlikely to have counteracted the vasoconstrictive effects of hypocapnia in the present investigation because the 15% O_2 inspired was not severe enough to cause increases in heart rate which are associated with coronary vasodilatation during hypoxia.

The effects of hypocapnia on the ECG in normal healthy subjects are not as noticeable as was previously thought. It is however, still of interest to see whether hypocapnia can provoke ischemic ECG changes in patients already suffering from reduced blood flow to the heart due to coronary artery disease.

Chapter 6

Does hypocapnia induced by mechanical hyperventilation cause clinically significant changes in the electrocardiogram of patients suffering from coronary artery disease?

6.1. Summary

The effect of hypocapnia on the ECG of patients suffering from coronary artery disease has not yet been satisfactorily assessed. In this pilot study, 16 patients have so far been identified as eligible to participate; however, only four have successfully completed the full experimental protocol. Of those four, two patients were taking long acting, isosorbide mononitrate medication (coronary vasodilator medication) which may have interfered with the effects of hypocapnia and were therefore considered in the medicated group A. The remaining two patients were not taking any medication known to interfere with the effects of hypocapnia and were grouped into the non-medicated group B. All patients were suffering from coronary artery disease with stable angina.

Hypocapnia did not cause any clinically significant ECG changes in any leads in the medicated group A. It was thought that the coronary vasodilator medication that these patients were taking may have interfered with the vasoconstrictive effects of hypocapnia and thus prevented a critical coronary occlusion.

In the non-medicated group B, hypocapnia caused an increase in T wave amplitude (of up to 0.05 ± 0.01mV [18%] in 3 leads) in patient 1B. In lead III, the increase in T wave amplitude resulted in the appearance of a hyperacute T wave. Hypocapnia also caused up sloping ST

segment depression in four anatomically contiguous leads (of up to 0.13 ± 0.02mV) in patient 2B. J point depression in these leads exceeded clinical thresholds for normality although it is possible that these ST segment changes were due to normal variations in the ECG.

These preliminary findings cannot confirm whether or not hypocapnia causes significant ECG changes in patients suffering from coronary artery disease. Conclusions about the effects of hypocapnia in these patients cannot be made until additional subjects are investigated. Therefore, further study is required to determine whether consistent changes can be induced by hypocapnia in patients who are not taking coronary vasodilator medication.

6.2. Introduction

As previously described (chapter 1.2.1, page 12), hypocapnia causes coronary vasoconstriction and decreased oxygen dissociation from haemoglobin which reduces O_2 delivery to the myocardium. In normal healthy subjects, this reduced O_2 delivery does not cause consistent, ischemic ECG changes of clinical importance (chapter 4, page 98). The aim of this investigation was to test whether hypocapnia causes clinically significant ECG changes in patients suffering from coronary artery disease with stable angina. If ECG changes are detected they could have important diagnostic implications. In patients who present with chest pain, it is necessary to perform a provocation test to reveal the existence of coronary artery disease (Braunwald *et al.*, 2001). Hypocapnia could potentially provoke myocardial ischemia by reducing coronary blood flow and decreasing O_2 dissociation from haemoglobin therefore causing an imbalance between O_2 demand and delivery.

6.2.1. Hypocapnia and the diagnosis of variant angina

Hypocapnia, induced by voluntary hyperventilation, causes significant ECG changes in patients suffering from a variant form of angina known as Prinzmetal's (Yasue *et al.*, 1978; Girotti *et al.*, 1982; Freeman & Nixon, 1985; Kruyswijk *et al.*, 1986; Ardissino *et al.*, 1987; Takaoka *et al.*, 1988; Fujii *et al.*, 1988; Weber *et al.*, 1988; Chelmowski & Keelan, 1988; Miyagi *et al.*, 1989). This form of angina occurs at rest or during normal activity and is characterized by the fact that it is not brought on by exercise, it is generally longer in duration and more severe than normal angina and it can be identified on an ECG by way of ST elevations with reciprocal depressions in leads corresponding to one specific coronary artery (Prinzmetal *et al.*, 1959). Prinzmetal's angina is brought on by a coronary artery spasm. Studies have shown that voluntary hyperventilation (for 2-8 minutes) can induce this coronary artery spasm resulting in both clinically significant ischemic ST segment changes (depression >1.5mV, elevation >2.0mV) and chest pain in 83-100% of patients tested (Girotti *et al.*, 1982; Previtali *et al.*, 1989; Freeman & Nixon, 1985; Kruyswijk *et al.*, 1986; Ardissino *et al.*, 1987; Takaoka *et al.*, 1988; Fujii *et al.*, 1988; Weber *et al.*, 1988; Chelmowski & Keelan, 1988; Miyagi *et al.*, 1989).

The mechanism that causes a coronary artery spasm in these patients is unclear. Possible causes have been proposed such as increased extracellular Ca^{2+} (Yasue *et al.*, 1978; Kruyswijk *et al.*, 1986; Ardissino *et al.*, 1987; Chelmowski & Keelan, 1988; Fujii *et al.*, 1988), hypersensitization of the coronary vascular bed to a normal physiological trigger of coronary artery spasm (Freeman & Nixon, 1985; Ardissino *et al.*, 1987; Chelmowski & Keelan, 1988) or a delayed response to respiratory alkalosis (Girotti *et al.*, 1982; Weber *et al.*, 1988).

Because voluntary hyperventilation causes consistent ECG changes in patients suffering from variant angina, it is considered an acceptable provocation test to be used in the diagnosis of this disease (Previtali *et al.*, 1989; Magarian & Mazur, 1991). It compares well with established diagnostic techniques such as ergonovine testing although its sensitivity is dependent on the severity of the disease. In patients who suffer from frequent spontaneous angina attacks, the hyperventilation test has a sensitivity of 96%, but in patients where attacks are less frequent, this may fall to as low as 55% (Previtali *et al.*, 1989). The effectiveness of this test is also affected by the degree of hypocapnic alkalosis that is induced by voluntary hyperventilation. Positive ECG changes should be accompanied by an arterial blood pH of >7.55 for variant angina to be positively identified (Magarian & Mazur, 1991). Voluntary hyperventilation is also useful in assessing the type of drug therapy to prescribe to patients with variant angina. Girotti *et al.*, (1982) showed that in patients with positive hyperventilation tests, the effectiveness of a variety of drug treatments could be assessed by performing a subsequent hyperventilation test following administration of individual drugs such as phentolamine, isosorbide dinitrate, propanolol, nifedipine and verapamil. Effective drugs were identified as those that produced a negative response to hyperventilation following drug administration.

6.2.2. Hypocapnia and the diagnosis of coronary artery disease with stable angina

The effects of hypocapnia on patients suffering from stable angina have been studied but findings are inconsistent because of the limitations associated with inducing hypocapnia with voluntary hyperventilation (chapter 1.2.3, page 18). Studies have demonstrated ischemic T wave changes (T wave inversion > -0.15mV) in up to 70% of patients suffering from coronary artery disease (Jacobs *et al.*, 1974; Joy & Trump, 1981; Kemp & Ellestad, 1968; Ardissino *et*

al., 1987; Lary & Goldschlager, 1974). 'False positive' results are widely reported by those using voluntary hyperventilation to detect coronary artery disease (Wasserberger *et al.*, 1956; Mchenry *et al.*, 1970; Lary & Goldschlager, 1974; Jacobs *et al.*, 1974; Evans & Lum, 1977; Joy & Trump, 1981).

Hypocapnia, induced by mechanical hyperventilation, eliminates the limitations associated with voluntary hyperventilation as previously described (chapter 1.2.2, page 17). Therefore, in the present investigation, mechanical hyperventilation was used with the digital ECG recording system described in chapter 2.9.2 (page 50) to examine the effects of hypocapnia on patients suffering from coronary artery disease with stable angina. The ECG was recorded and compared to recent AHA, ACCF and HRS clinical thresholds for myocardial ischemia (Kenigsberg *et al.*, 2007; Wagner, 2008; 2009; Rautaharju *et al.*, 2009).

6.3. Methods

Sixteen patients suffering from coronary artery disease were identified as eligible to participate, however, only four (aged 61 ± 8 years old [42-74 years old]) (4 male) consented to participate in the full experimental protocol. All four patients had normal resting ECGs but had a positive exercise stress test. All four patients had undergone a coronary angiogram to determine the extent of their disease (see figures 6.1 and 6.2 for details) and were awaiting either coronary angioplasty or a coronary artery bypass graft after participation in the experiment was complete. Patients gave informed consent and experiments were approved by the Walsall Local Research Ethics Committee. All experiments were performed in accordance with the Declaration of Helsinki as stated by the American Physiological Society (2002). All

patients were given a medical examination prior to participation in the study by the consultant cardiologist. Full patient details and past medical history can be seen in appendix.

Initially, patients taking long acting isosorbide mononitrate medication (coronary vasodilator medication) were included in the study. However, after no obvious ECG changes were seen in two patients it was decided by the consultant cardiologist that patients taking this type of medication should be excluded as it may interfere with the effects of hypocapnia. Data from two of these patients has been included in this report and is considered in the 'medicated' group A. To allow those on long acting nitrate medication to participate in the study, ethical approval was obtained to ask all those receiving nitrate treatment to refrain from taking their medication for a period of 36 hours prior to the experimentation procedure. Two patients not taking any medication thought to interfere with this investigation were grouped into the 'non-medicated' group B.

Coronary artery disease affected different arteries in each patient and thus each was considered separately. The region and vessel affected by the coronary artery disease dictates the ECG lead in which ischemic changes occur. Details of the patient's disease and the ECG leads in which changes during provocation of angina were expected are presented in table 6.1 (medicated group A), table 6.2 (non-medicated group B) and figures 6.1-6.2 (which show the location of vessel occlusion in each subject).

For each trial, continuous blood pressure was recorded using a finger plethysmograph (Finapres 2300) and end-tidal CO_2 was recorded with an in-line capnograph (Hewlett Packard 78354A). O_2 saturation was monitored continuously using a pulse oximeter (N-200 series

oximeter, Nellcor) as a safety precaution. A continuous 7 lead ECG (6 limb leads, 1 precordial lead) was recorded using a portable patient monitor (Philips IntelliVue, MP30) to allow instantaneous ST segment monitoring throughout the experiment.

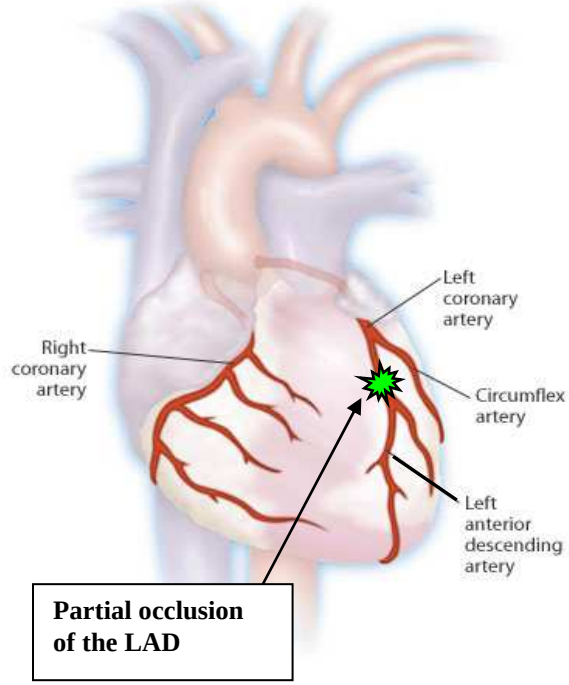
Table 6.1. Group A (medicated) - Patient's related medication, disease type, disease location and affected ECG leads.

Patient/ Disease type	**Coronary vasodilator medication**	**Affected vessel**	**Affected region of the heart**	**Affected ECG leads**
1A. Single vessel disease	Long acting nitrates	LAD **(see figure 6.1a)**	Extensive anterior Anteroseptal	I, aVL, V_{1-6}
2A. Triple vessel disease	Long acting nitrates	LAD LCX RCA **(see figure 6.1b)**	Extensive anterior Anteroseptal Lateral Inferolateral	I, II, III, aVL, aVF, V_{1-6}

Table 6.1. LAD = left anterior descending coronary artery. LCX = left circumflex artery. RCA = right coronary artery.

Figure 6.1. Group A (medicated) – location of coronary artery disease identified on the coronary angiogram

a) Patient 1A (single vessel disease)



b) Patient 2A (triple vessel disease)

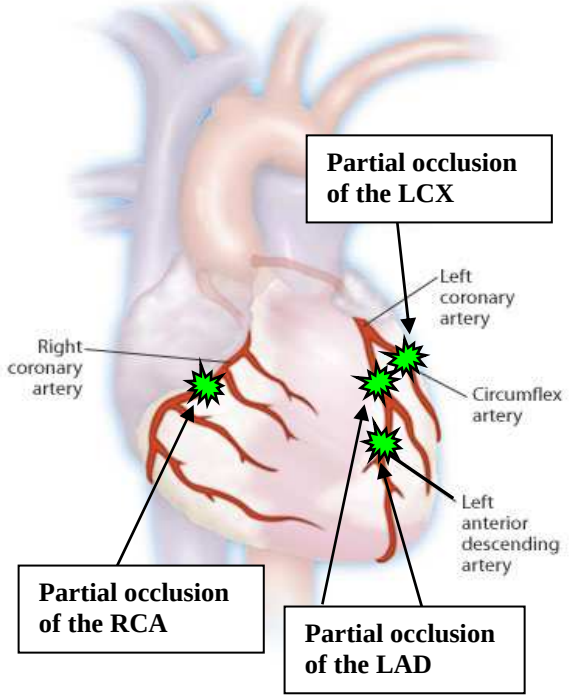


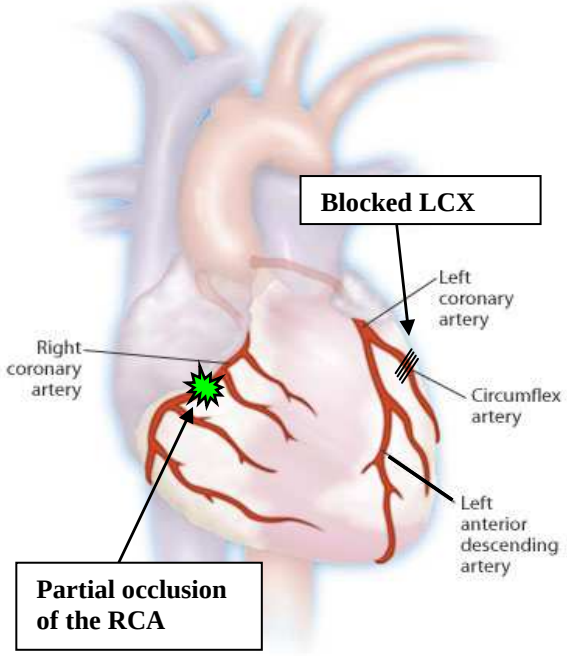
Table 6.2. Group B (non-medicated) - Patient's related medication, disease type, disease location and affected ECG leads.

Patient/ Disease type	**Coronary vasodilator medication**	**Affected vessel**	**Affected region of the heart**	**Affected ECG leads**
1B. Double vessel disease	None	LCX RCA **(see figure 6.2a)**	Lateral Inferolateral	II, III, aVF, V_{1-2}
2B. Double vessel disease	None	Left main LAD **(see figure 6.2b)**	Extensive anterior Anteroseptal	I, II, III, aVL, aVF, V_{1-6}

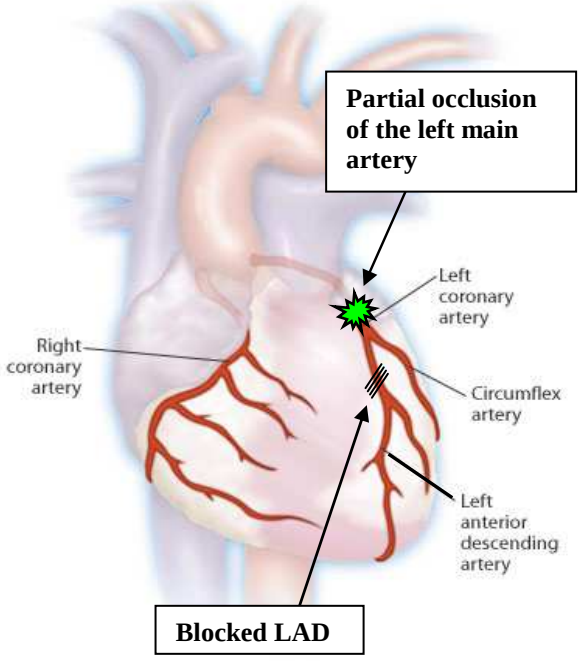
Table 6.2. LAD = left anterior descending coronary artery. LCX = left circumflex artery. RCA = right coronary artery. Left main = main left coronary artery

Figure 6.2. Group B (non-medicated) – location of coronary artery disease identified on the coronary angiogram

a) Patient 1B (double vessel disease)



b) Patient 2B (double vessel disease)



Mechanical hyperventilation was used to induce normocapnia and hypocapnia, in two trials as previously described in chapter 2.3 (page 26). The order and duration of normocapnia and hypocapnia can be seen in figure 4.1 (page 107). A 12 lead ECG was recorded for two minutes at the end of each condition (figure 4.1). T wave amplitude and ST segment height were measured in each lead and compared to AHA, ACCF and HRS gender and age specific clinical thresholds for myocardial ischemia (tables 2.3 [page 60] and 2.4 [page 61]) (Rautaharju *et al.*, 2009; Surawicz *et al.*, 2009; Wagner *et al.*, 2009; Wagner, 2008).

A positive result in the 'diagnostic test' was defined by a T wave elevation/inversion and/or ST segment elevation/depression in two more, anatomically contiguous ECG leads associated with the known affected region of the myocardium (identified by coronary angiography). All data are expressed as means ± standard error for each patient across both trials. ECG leads affected by coronary artery disease (identified by the coronary angiogram) are indicated on each graph by the grey boxes. A 2-tail paired t-test was performed to compare heart rate and blood pressure in normocapnia and hypocapnia. Statistical analysis was not performed on individual patient's ECG data because each was examined with respect to their specific disease state.

Figure 6.3. In all 4 patients $PetCO_2$ decreased by 17 mmHg during hypocapnia and heart rate and mean arterial pressure remain unchanged

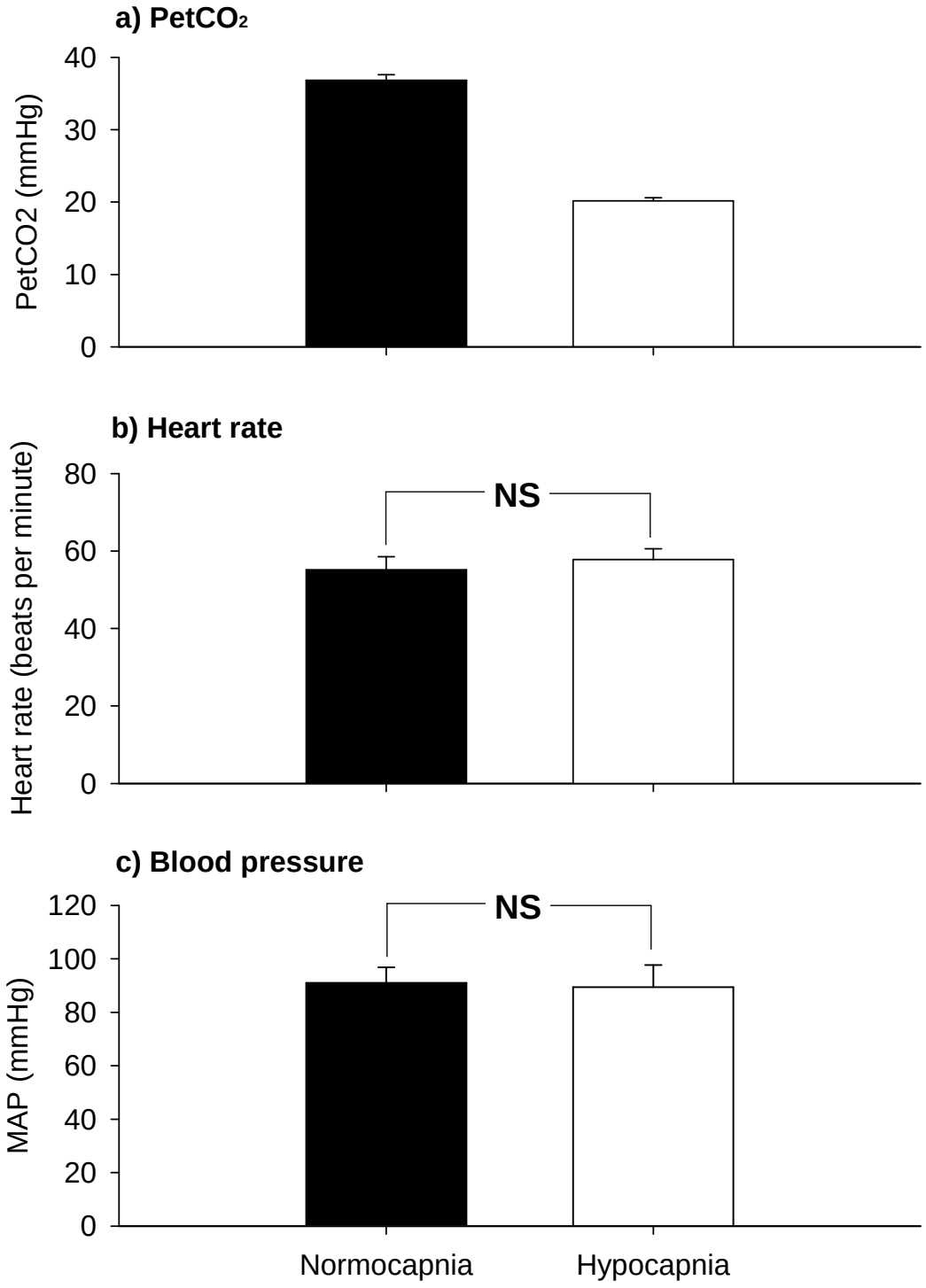


Figure 6.3. Mean ± SE $PetCO_2$, heart rate and mean arterial pressure (MAP) changes during mechanical hyperventilation in normocapnia and hypocapnia in 4 patients. All values in hypocapnia, *NS* vs. normocapnia.

Figure 6.3 shows that in all patients, mechanical hyperventilation in hypocapnia caused a mean decrease in $PetCO_2$ of 17 ± 0 mmHg but had no affect on heart rate or blood pressure.

6.4.1. ECG changes in the medicated group A

Patient 1A (medicated group)

In patient 1A, hypocapnia caused an increase in T wave amplitude in leads V_{1-3} (increase of 0.03 ± 0.01mV [20%] in V_1, increase of 0.07 ± 0.01mV [15%] in V_2, increase of 0.03 ± 0.04mV [7%] in V_3) but wave amplitudes did not exceed clinically significant thresholds (figure 6.4). No ST segment elevation or depression was seen in any of the 12 leads of the ECG (figure 6.5).

Patient 2A (medicated group)

Hypocapnia did not cause clinically significant T wave changes in any of the 12 leads of the ECG (figure 6.6). In addition, hypocapnia did not cause any clinically significant ST segment deviation in any of these leads. The largest ST segment change was depression of 0.07 ± 0.02mV in lead V_4 (figure 6.7) but this remained within clinical limits for normality.

Figure 6.4. In patient 1A (medicated group), hypocapnia did not cause clinically significant T wave elevation in any of the frontal or transverse plane leads of the ECG

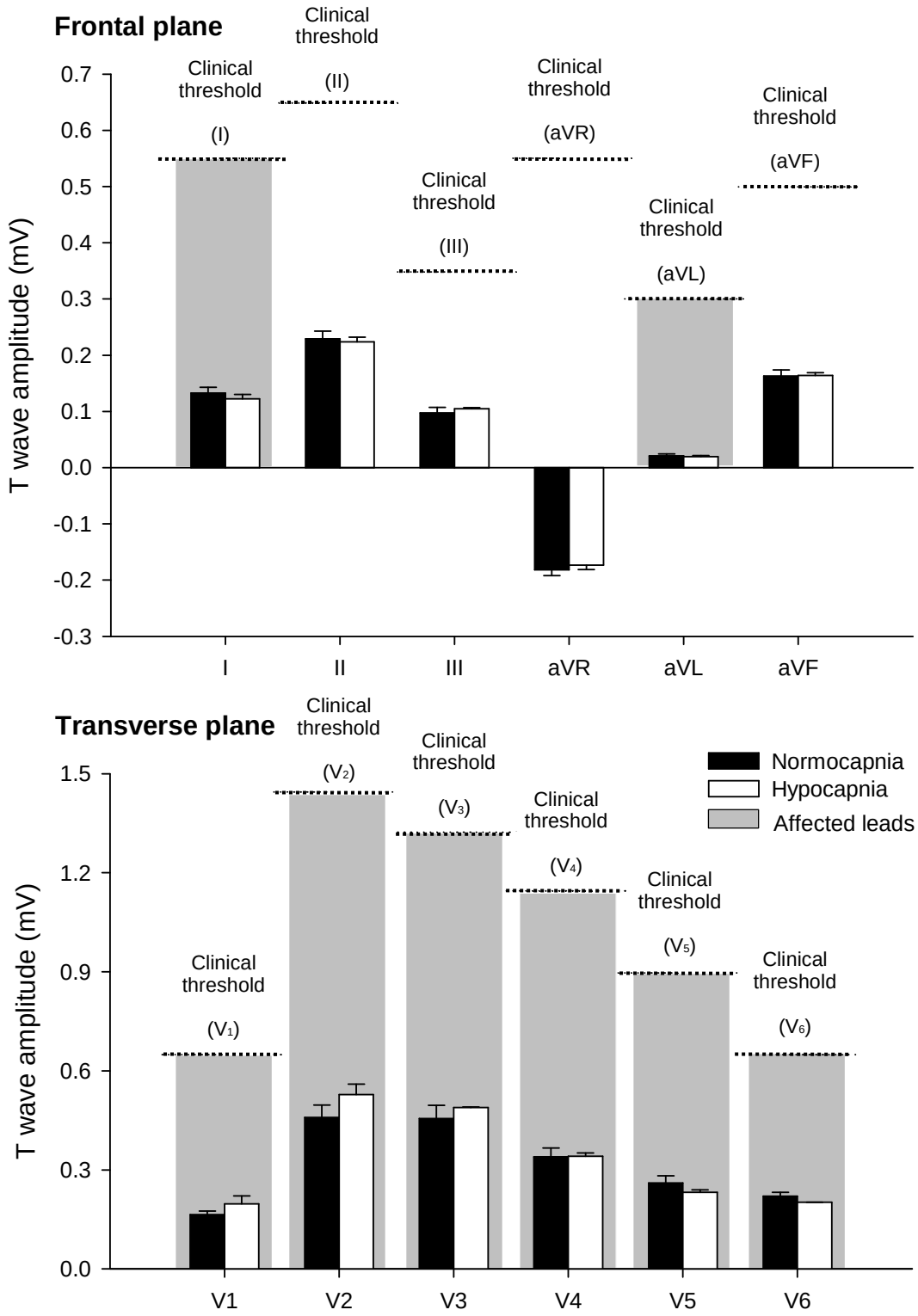


Figure 6.4. Mean ± SE of 2 trials. T wave amplitudes during normocapnia and hypocapnia in patient 1A. Wave amplitudes above the dotted line are considered of clinical importance. Relevant leads are highlighted by grey boxes.

Figure 6.5 In patient 1A (medicated group), hypocapnia did not cause significant ST segment changes in any of the 12 leads of the ECG

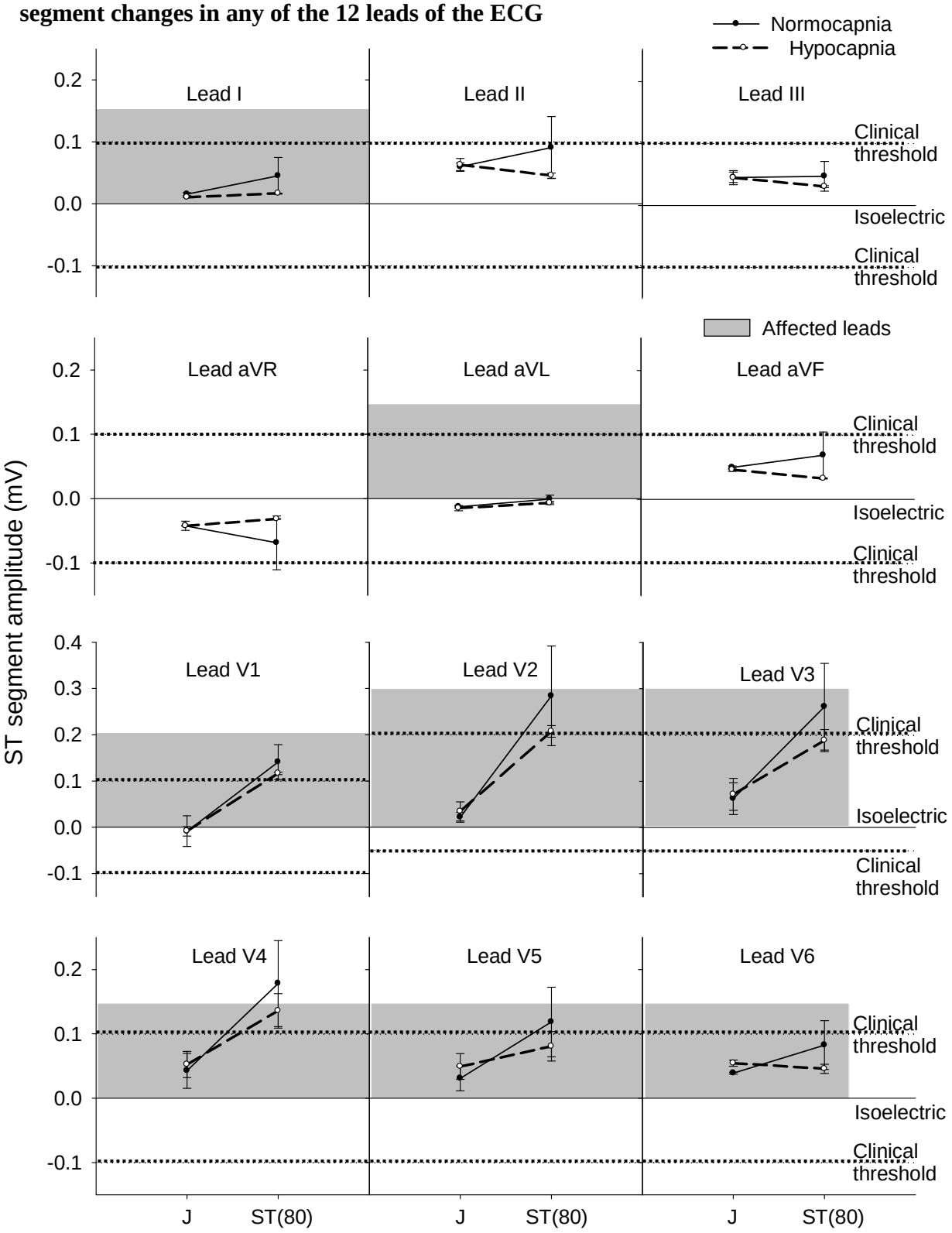


Figure 6.5. Mean ± SE of 2 trials. ST segments are defined by deviation of the J point from the isoelectric line. The extent of these changes is estimated by deviation of the ST(80) point. Data points above/below the dotted line represent clinically significant deviation from isoelectric (straight line). Relevant leads are indicated by the grey boxes

Figure 6.6. In patient 2A (medicated group), hypocapnia did not cause clinically significant T wave elevation in any of the frontal or transverse plane leads of the ECG

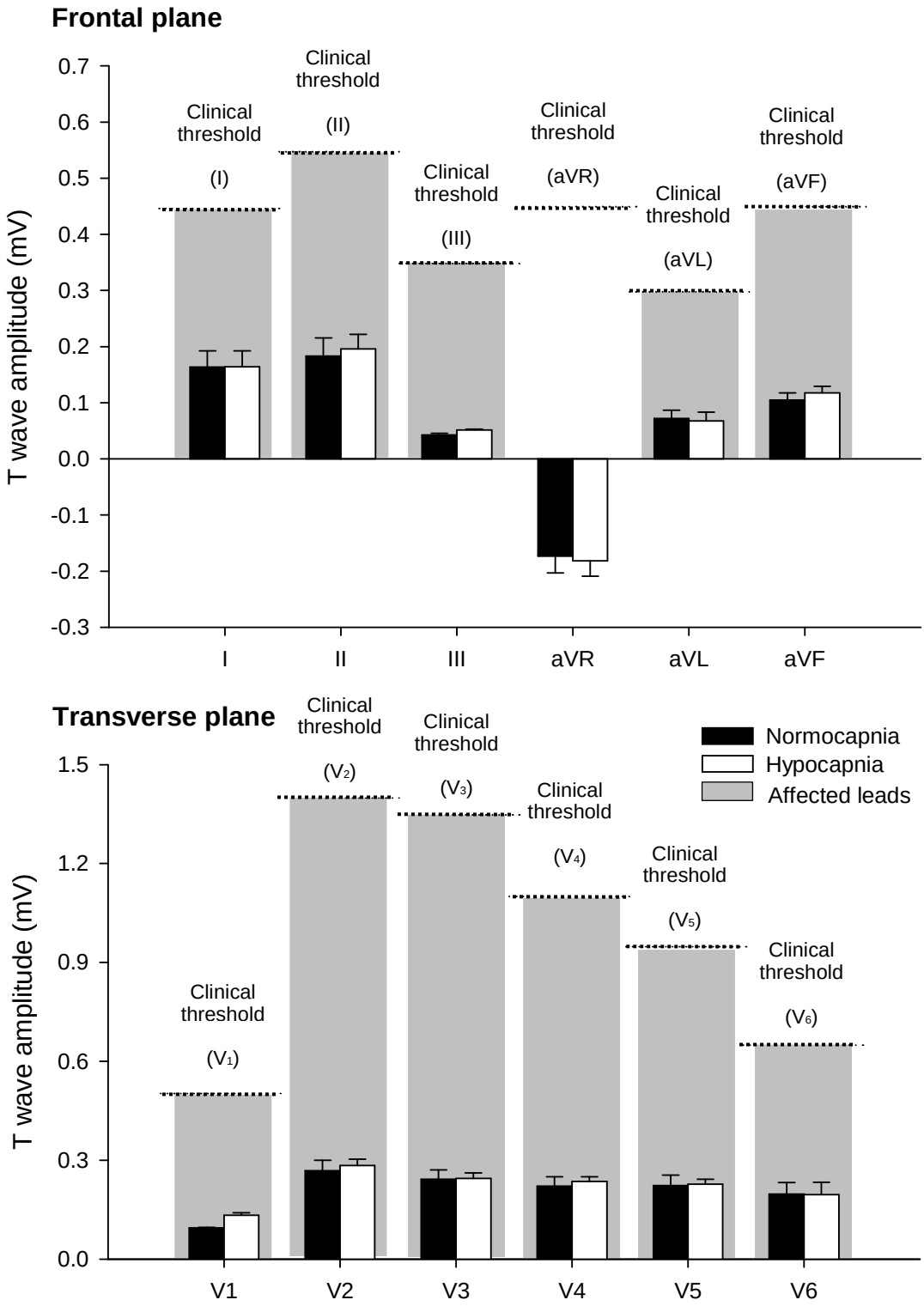


Figure 6.6. Mean ± SE of 2 trials. T wave amplitudes during normocapnia and hypocapnia in patient 2A. Wave amplitudes above the dotted line are considered of clinical importance. Relevant leads are highlighted by grey boxes.

Figure 6.7. In patient 2A (medicated group), hypocapnia did not cause significant ST segment changes in any of the 12 leads of the ECG

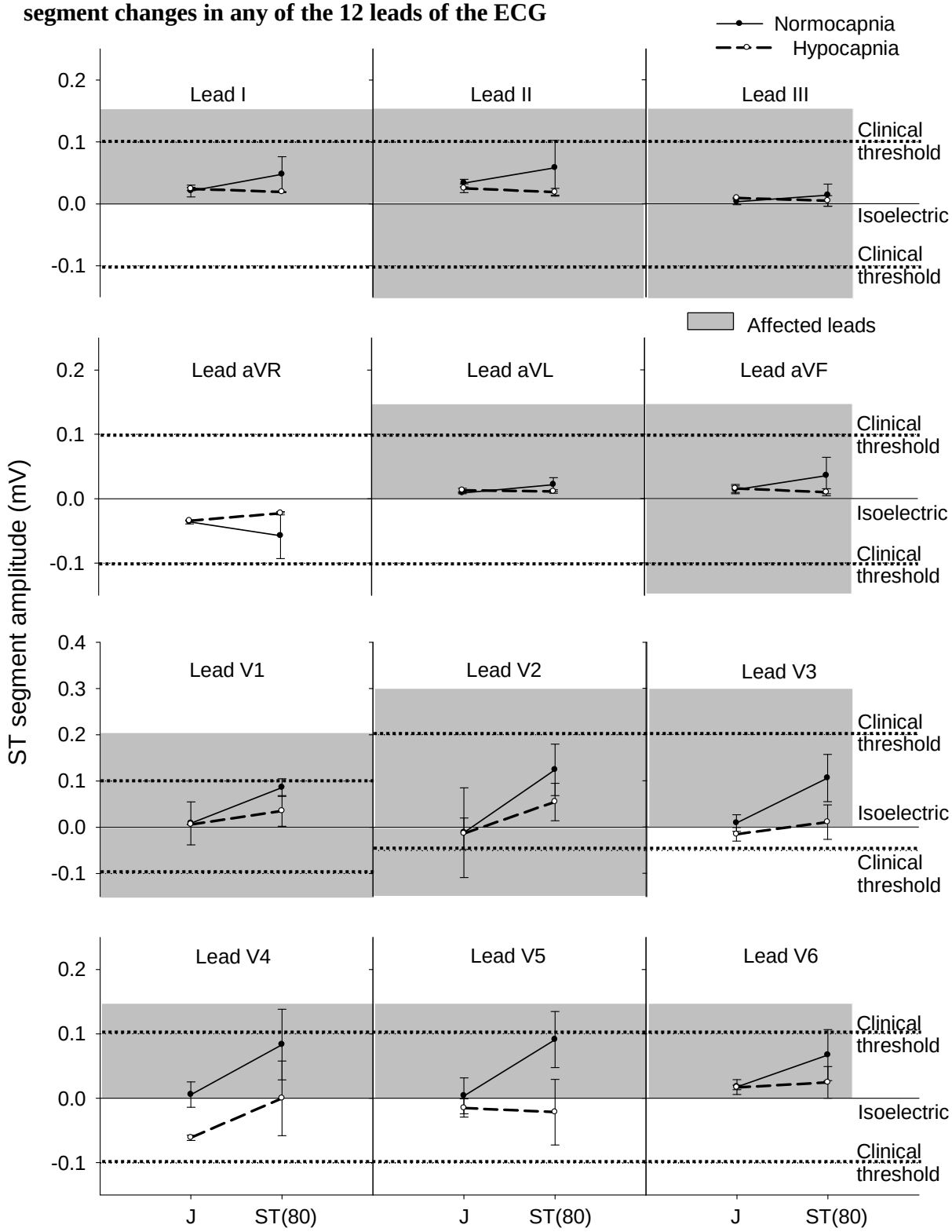


Figure 6.7. Mean ± SE of 2 trials. ST segments are defined by deviation of the J point from the isoelectric line. The extent of these changes is estimated by deviation of the ST(80) point. Data points above/below the dotted line represent clinically significant deviation from isoelectric (straight line). Relevant leads are indicated by the grey boxes

6.4.1. ECG changes in the non-medicated group B

Patient 1B (non-medicated group)

Hypocapnia caused an increase in T wave amplitude in leads II (increase of 0.04 ± 0.02mV [11%]), III (increase of 0.05 ± 0.01mV [18%]) and aVF (increase of 0.05 ± 0.01mV [15%]) (figure 6.8). The absolute T wave amplitude in lead III did exceed clinical thresholds for ischemia suggesting that it had become hyperacute during hypocapnia. Figure 6.9 shows that T wave elevation was not accompanied by ST segment changes in any of the expected leads.

Patient 2B (non-medicated group)

Figure 6.10 shows that hypocapnia caused no changes in T wave amplitude in any clinically relevant leads. Hypocapnia did not cause any clinically significant ST segment elevation in the affected ECG leads (figure 6.11). However, a small ST depression did occur during hypocapnia in leads V_2 (-0.06 ± 0.01mV), V_3 (-0.11 ± 0.01mV), V_4 (-0.13 ± 0.02mV) and V_5 (-0.11 ± 0.01mV) which was beyond the clinical thresholds of -0.05 to -0.10mV (figure 6.11). ST segment depression in these leads was not accompanied by ST flattening or depression of the ST(80) point.

Figure 6.8. In patient 1B (non-medicated group), hypocapnia causes clinically significant T wave elevation in lead III on the frontal plane of the ECG

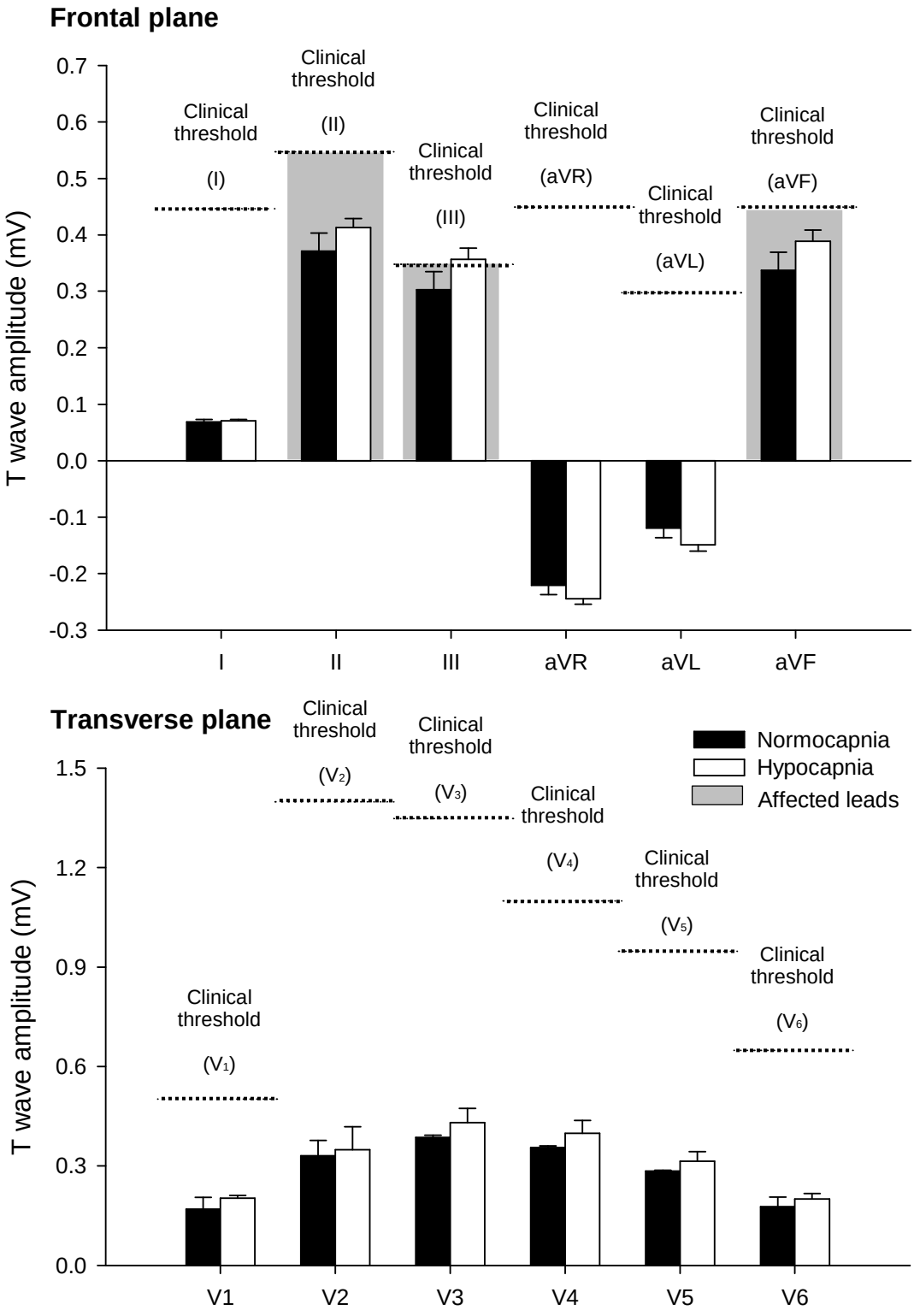


Figure 6.8. Mean ± SE of 2 trials. T wave amplitudes during normocapnia and hypocapnia in patient 1B. Wave amplitudes above the dotted line are considered of clinical importance. Relevant leads are highlighted by grey boxes.

Figure 6.9. In patient 1B (non-medicated), hypocapnia did not cause significant ST segment changes in any of the 12 leads of the ECG

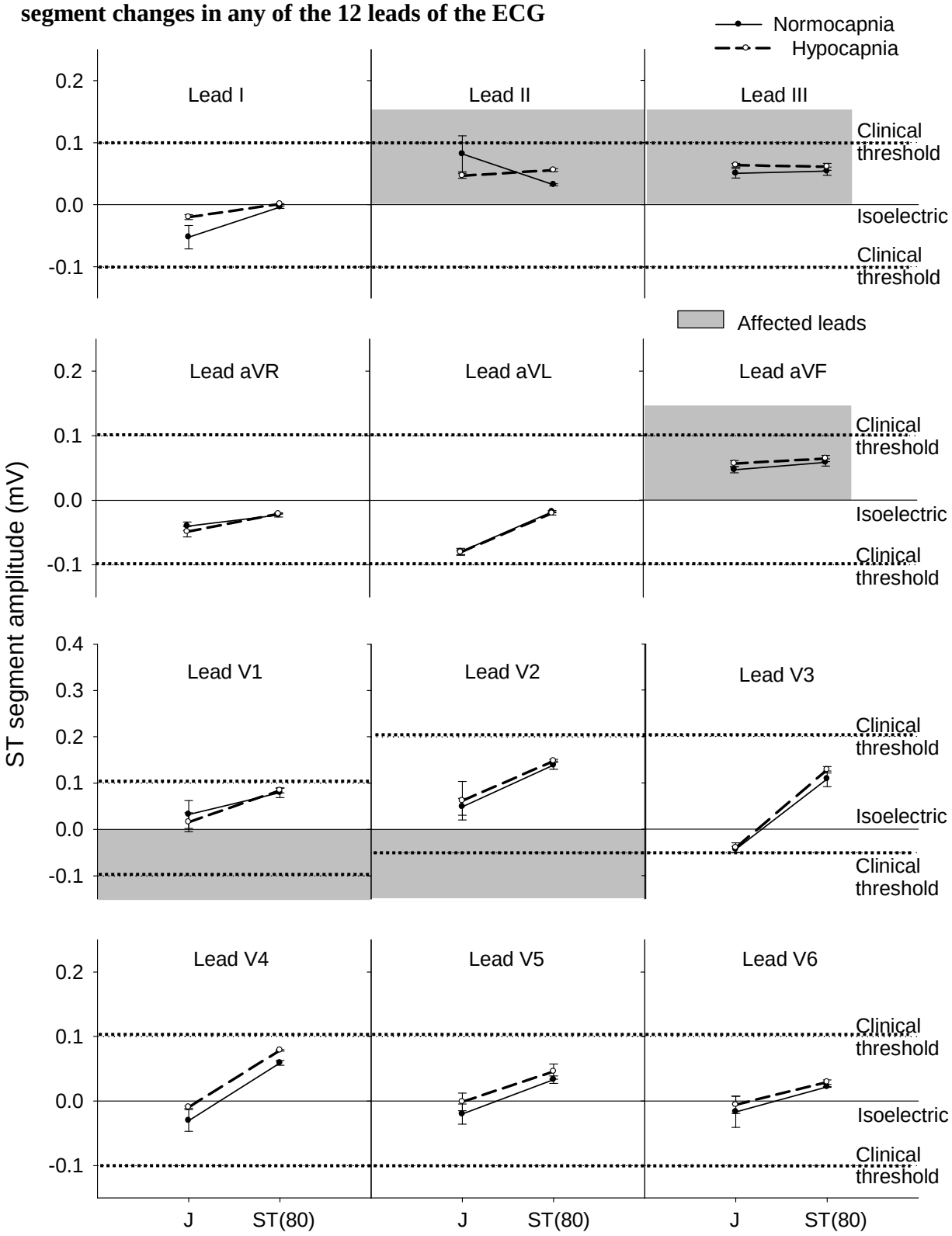


Figure 6.9. Mean ± SE of 2 trials. ST segments are defined by deviation of the J point from the isoelectric line. The extent of these changes is estimated by deviation of the ST(80) point. Data points above/below the dotted line represent clinically significant deviation from isoelectric (straight line). Relevant leads are indicated by the grey boxes

Figure 6.10. In patient 2B (non-medicated), hypocapnia did not cause clinically significant T wave elevation in any of the frontal or transverse plane leads

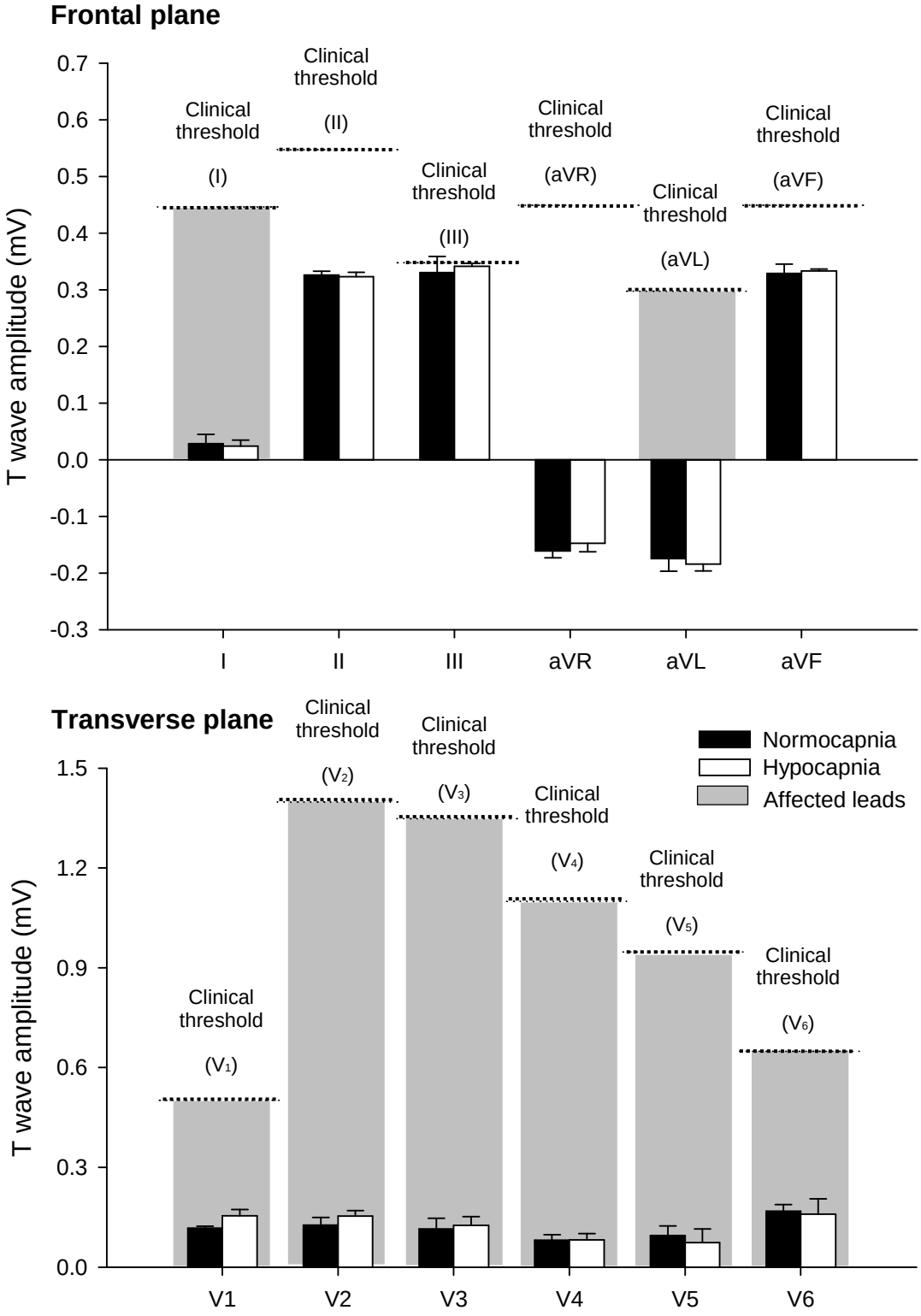


Figure 6.10. Mean ± SE of 2 trials. T wave amplitudes during normocapnia and hypocapnia in patient 2B. Wave amplitudes above the dotted line are considered of clinical importance. Relevant leads are highlighted by grey boxes.

Figure 6.11. In patient 2B (non-medicated group), hypocapnia causes ST segment changes in leads $V_{2\text{-}5}$ of potential clinical importance

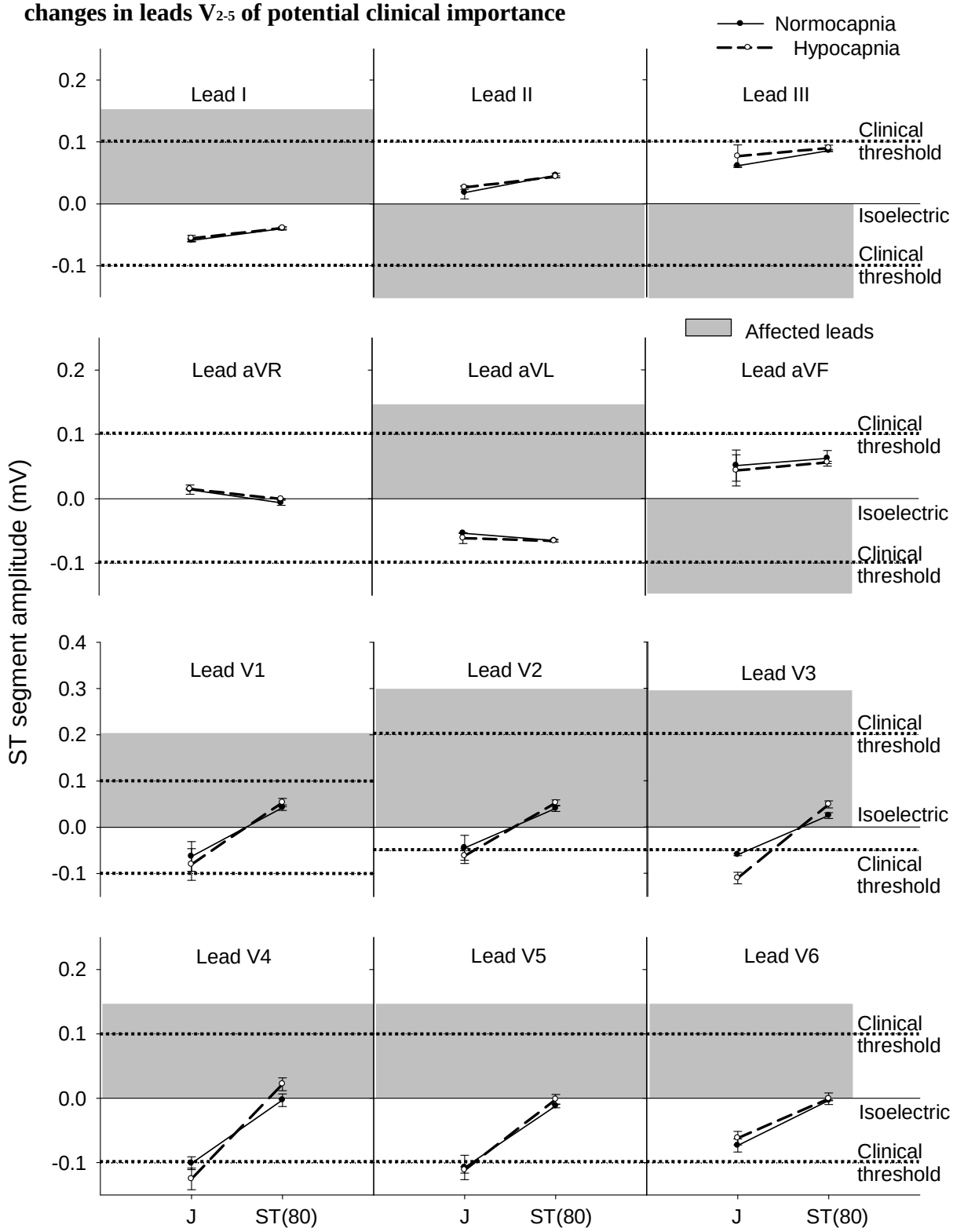


Figure 6.11. Mean ± SE of 2 trials. ST segments are defined by deviation of the J point from the isoelectric line. The extent of these changes is estimated by deviation of the ST(80) point. Data points above/below the dotted line represent clinically significant deviation from isoelectric (straight line). Relevant leads are indicated by the grey boxes

6.5. Discussion

In the present investigation, patients in the medicated group A did not display any consistent or clinically significant ECG changes during hypocapnia induced by mechanical hyperventilation. The absence of changes could be attributed to either a lack of effect of hypocapnia on cardiac electrical activity or isosorbide mononitrate medication interfering with this effect. In the non-medicated group B, hypocapnia caused small changes in T wave amplitude and ST segment height. Absolute T wave amplitudes in lead III during hypocapnia in one patient were beyond clinical limits for normality. In another patient, up sloping ST segment depression was seen in four anatomically contiguous leads. This was beyond clinical thresholds for normality although the actual change from normocapnia was small and depression of the J point was not accompanied by depression of the ST(80) point. These changes do not confirm the presence of myocardial ischemia during hypocapnia in patients suffering from coronary artery disease. However, investigations in further patients are needed before any definitive conclusions can be made.

6.5.1. Absence of ECG changes in group A (medicated)

In both patients in the medicated group A, hypocapnia did not cause any clinically significant ECG changes. In these patients, it is possible that the lack of changes were due to the nitrate medication they were taking. Isosorbide mononitrate medication stimulates the production of nitric oxide in the coronary arteries which relaxes the vascular smooth muscle causing vasodilation (Braunwald *et al.*, 2001). It is possible that the vasodilatory effects of isosorbide mononitrate may have counteracted the vasocostrictive effects of hypocapnia, thus preventing the critical coronary stenosis necessary to reduce O_2 delivery to the myocardium.

Alternatively, the lack of ECG changes seen in this medicated group A may simply represent the lack of effect that hypocapnia has on the ECG in patients suffering from coronary artery disease. Whilst previous studies suggest that hypocapnia reduces blood flow and O_2 delivery to the myocardium (Rowe *et al.,* 1962; Karlsson *et al.*, 1994; Laffey & Kavanagh, 2002; Neill & Hattenhauer, 1975; Yokoyama *et al.*, 2008), none have comprehensively proven that this is sufficient to cause consistent ECG changes in patients suffering from coronary artery disease. To the contrary, Kazmaier *et al.,* (1998) suggested that in patients suffering from coronary artery disease, hypocapnia (31mmHg) has no effect on coronary blood flow although in this case the stimulus may not have been severe enough to cause an effect. It is possible that in some patients, diseased arteries do not respond the stimuli of low $PaCO_2$ that causes coronary vasoconstriction in the same way as normal healthy arteries. Without knowledge of the mechanism which causes coronary vasoconstriction, it is difficult to speculate as whether or not this is the case.

Following the study of these patients in the medicated group A, it was decided by the consultant cardiologist that any other patients taking long acting nitrate medication either refrain from taking their medication during testing periods or should be excluded from the study.

6.5.2. T wave changes in group B (non-medicated)

In those patients not taking nitrate medication, hypocapnia did cause small ECG changes that could be of clinical interest. During hypocapnia, increases in T wave amplitude of up to 0.05 ± 0.01mV occurred in leads II, III and aVF in patient 1B. This resulted in the appearance of a hyperacute T wave in lead III. This patient suffered from double vessel coronary artery

disease of the left circumflex artery and right coronary artery. This hyperacute T wave in lead III would therefore be consistent with the occurrence myocardial ischemia due to insufficient O_2 delivery from the right coronary artery. If this were the case, hyperacute T waves would also be expected in leads II and aVF. However, consistent changes of this type were not seen.

Previous studies have also failed to produce consistent T wave changes during hypocapnia induced by voluntary hyperventilation. In 114 patients, Kemp & Ellestad (1968) reported 11 cases (10%) where voluntary hyperventilation caused ischemic T wave changes. Others have demonstrated T wave changes in patients suffering from coronary artery disease (diagnosed by coronary angiography) during voluntary hyperventilation in up to 70% of patients (Jacobs *et al.*, 1974; Joy & Trump, 1981; Ardissino *et al.*, 1987). Lary & Goldschlager (1974) found that 7/192 patients (3%) with coronary artery disease, diagnosed by coronary angiography, exhibit ischemic T wave changes during voluntary hyperventilation. In the present investigation, it is impossible to draw conclusions from T wave changes seen in one non-medicated patient, particularly as hyperacute T waves were not seen in all the relevant ECG leads.

6.5.3. ST segment changes in group B (non-medicated)

Clinically significant ST segment elevation was not seen in any patients during hypocapnia. Significant depression of the ST segment (at the J point) was seen during hypocapnia in the non-medicated patient 2B in leads V_{2-5} (figure 6.11). The importance of these changes is unclear because J point depression was not accompanied by depression of the ST(80) point (ST flattening) and the largest absolute decrease in ST height between normocapnia and hypocapnia was only 0.05 ± 0.01mV.

ST segment depression in leads V_{2-5} indicates the existence of subendocardial ischemia in the anteroseptal and extensive anterior regions of the heart. Subendocardial ischemia is usually caused by increased oxygen demand due to increased myocardial O_2 demand (Wagner, 2008). In the present study, the patient's heart rate and blood pressure (and therefore myocardial O_2 demand) remained normal throughout the experiment. The observed ECG changes were not consistent with a reduction in blood supply caused by the partially occluded left main coronary artery or the blocked LAD (as indicated by the coronary angiogram). It therefore seems unlikely that the observed changes are of clinical importance, particularly as ST segment flattening did not occur and up sloping ST segment depression often occurs due to normal variation of the ECG (Wagner, 2008).

6.5.4. Conclusions

The effects of hypocapnia in patients suffering from coronary artery disease remain inconclusive due to the small number of subjects recruited in this pilot study. In those patients who were investigated, it is not clear whether prescribed medication may have interfered with the effects of hypocapnia on the ECG.

Ideally, it is best to recruit patients with established coronary artery disease (diagnosed by coronary angiography) that have not undergone any surgery and are not taking any medication. Where this was not possible, an attempt was made to identify medications most likely to interfere with the results of this investigation and recruit patients who are not taking them. It cannot be denied that other medications may have interfered with the results of this study via unknown interactions with hypocapnia and its effects on coronary vasoconstriction.

Consistent ECG changes induced by hypocapnia in patients suffering from coronary artery disease are yet to be demonstrated using mechanical hyperventilation. This pilot study shows that it is possible to investigate this if patients can be identified and conflicting medication can be properly managed. A full investigation of the effects of hypocapnia on the ECG in patients suffering from single, double or triple vessel coronary artery disease is necessary to establish whether this technique of inducing hypocapnia by mechanical hyperventilation has any clinical value in the early diagnosis of coronary artery disease.

Chapter 7

General Discussion

7.1. Summary of findings

This thesis investigation set out to identify whether hypocapnia causes significant changes in the 12 lead ECG of normal subjects and whether these changes are exaggerated in patients suffering from coronary artery disease. This was done using an established method of mechanical hyperventilation (Cooper *et al.*, 2004) in which hypocapnia can be induced without altering breathing frequency and tidal volume. An updated and improved version of the ECG apparatus used by Rutherford *et al.,* (2005) was utilised so that 12 ECG leads could be recorded simultaneously and the effects of hypocapnia on cardiac electrical activity could be examined.

This investigation confirms that mechanical hyperventilation is an effective method for inducing severe, sustained hypocapnia which is well tolerated by both normal healthy subjects and patients suffering from coronary artery disease. In all studies, $PetCO_2$ was consistently reduced to 20 ± 0mmHg and kept at this level for $\geq$10 minutes in all subjects. Mechanical hyperventilation itself was well tolerated by all subjects, particularly in those accustomed to a hospital setting.

The main finding of this thesis investigation is that hypocapnia, induced by mechanical hyperventilation, does not cause consistent ECG changes indicative of myocardial ischemia in normal healthy subjects. The findings of a pilot study examining the effects of hypocapnia on

patients suffering from coronary artery disease were inconclusive; however, preliminary results suggest that there is little effect on the ECG. Studies using echocardiography to assess the heart function also failed to show any effects of hypocapnia in normal healthy subjects. It therefore seems unlikely that ECG changes (in normal subjects) during hypocapnia are due to a reduction in O_2 availability in the myocardium as has been previously suggested (Rutherford *et al.*, 2005). These findings question the degree to which hypocapnia causes a reduction in O_2 delivery to the myocardium via coronary vasoconstriction and an increased affinity of haemoglobin for O_2 as proposed by Neill & Hattenhauer (1975).

The reasons for the discrepancy between the findings of this thesis investigation and those of Rutherford *et al.,* (2005) are unclear. Both studies use the same method of inducing hypocapnia with mechanical hyperventilation in a similar number of normal healthy subjects (18 in the present investigation vs. 15 studied by Rutherford *et al.,* 2005). The main difference between the two studies was the ECG apparatus used. Both were custom made for each experiment to enable accurate analysis of the ECG waveform. In the present investigation, the ECG apparatus used recorded a 12 lead ECG simultaneously and was calibrated against a patient simulator calibration box (Dynatech Nevada Inc., Model 212B, Nevada, USA) and a standard digital ECG machine (Philips Hewlett Packard Pagewriter 200). In the study by Rutherford *et al.,* (2005), a single lead ECG was recorded using an analogue Neurolog NL840 amplifier calibrated against a custom built 200uV generator. T wave amplitudes at rest recorded by Rutherford *et al.,* (2005) were 0.73mV, more than double the size of those in the present study (0.32mV). Resting T wave amplitudes recorded using the ECG apparatus of Rutherford *et al.,* (2005) would be considered clinically abnormal (hyperacute) if they were compared to the clinical thresholds used in the present study (>0.5mV [Wagner, 2008]).

A secondary aim of this thesis investigation was to quantify the degree to which electrode modification alters wave amplitudes in the 12 lead ECG. Electrode modification was found to significantly decrease R and T wave amplitudes in Lead I of the ECG by 20% at rest and during mechanical hyperventilation. Rutherford *et al.,* (2005) used the modified electrode placement in experiments studying the effects of hypocapnia on the ECG. Even with a 20% reduction in T wave amplitude (which would have occurred had they used the standard electrode placement), the resulting T wave amplitude (0.58mV) would still have exceeded clinical thresholds for hyperacute T waves (>0.5mV [Wagner, 2008]). This further emphasises the notion that the ECG apparatus used by Rutherford *et al.,* (2005) study did not produce an accurate representation of the ECG waveform. Because the ECG apparatus used in the present investigation was calibrated against two separate machines commonly used in clinical practice (Dynatech patient simulator calibrator box and Philips Hewlett Packard Pagewriter 200), ECGs recorded were considered accurate and comparable to clinical guidelines.

It is unclear where the threshold of 0.1mV for a clinically important reduction in T wave amplitude was acquired by Rutherford *et al.,* (2005). Up to date guidelines (Rautaharju *et al.*, 2009; Surawicz *et al.*, 2009; Wagner *et al.*, 2009) and textbooks (Wagner, 2008) suggest that changes in the amplitude of the T wave are only of clinical importance if a change in the polarity of the T wave occurs (a positive T wave becomes inverted or *vice versa*) or the absolute amplitude is above 0.5mV (in the frontal plane) or 1.0mV (in the transverse plane). Since the findings of Rutherford *et al.,* (2005) were not repeatable in the present investigation and no evidence can be found to support the claims that their findings were of clinical importance, it appears likely that their observed changes in T wave amplitude during

hypocapnia were attributable to random variations rather than a decrease in O_2 availability in the myocardium.

7.2. Clinical Implications

This thesis investigation shows that hypocapnia does not cause consistent ischemic ECG or echocardiographic changes in normal health subjects and is unlikely to in patients suffering from coronary artery disease. It is possible that a more severe hypocapnia might be more effective at producing ischemic ECG changes in both patients and normal subjects. However, the side effects that accompany the reduction of $PetCO_2$ below 20 mmHg (such as tetany and paraesthesia, Macefield & Burke, 1991) mean that it would not be appropriate to perform this in a clinical setting or in normal healthy volunteers.

The implications of these findings suggest that the technique of mechanical hyperventilation has less clinical value in the diagnosis of coronary artery disease than was previously thought. Had the induction of hypocapnia using mechanical hyperventilation caused more exaggerated ECG changes in patients suffering from coronary artery disease than those observed in normal healthy subjects then the technique may have been of some value as a provocation test for stable angina (Rutherford *et al.*, 2005). However, this thesis investigation, including a pilot study in patients suffering from coronary artery disease, suggests that this was not the case and that mechanical hyperventilation is of little clinical value. It should be noted that the pilot study in patients was conducted in a small population of four patients, two of whom were taking medication which may have interfered with the effects of hypocapnia on coronary vasoconstriction. Therefore, it may be premature to make conclusions about the clinical implications of this technique in patients suffering from coronary artery disease.

7.3. Limitations

Likelihood of myocardial ischemia

Initially, it is worth considering the likelihood that hypocapnia can induce myocardial ischemia in normal healthy subjects. Existing evidence suggests that hypocapnia causes a reduction in coronary blood flow (Rowe *et al.,* 1962; Neill & Hattenhauer, 1975; Yokoyama *et al.*, 2008) and an increased affinity of haemoglobin for O_2 (Neill & Hattenhauer, 1975). It has been suggested that this causes a reduction in O_2 delivery to the myocardium which is reflected, even in normal healthy subjects, by ischemic changes in the T wave during mechanical hyperventilation in hypocapnia (Rutherford *et al.,* 2005). Previous literature states that a coronary artery must be occluded by at least 30-60% for blood flow to become significantly reduced (May *et al.,* 1963; Braunwald *et al.*, 2001). However, even an occlusion of 60% will not necessarily result in myocardial ischemia. In the presence of coronary artery disease, current NICE guidelines suggest that an angina attack (myocardial ischemia) is only likely if the coronary artery is occluded by >70% (Cooper *et al.*, 2010). It seems unlikely that hypocapnia would cause an occlusion of >70% and resulting myocardial ischemia in a subject with healthy coronary arteries, even with the additional effects of reduced O_2 dissociation due decreased $PaCO_2$.

In light of the present investigation, which highlights the inaccuracies of the findings by Rutherford *et al.,* (2005), the notion that hypocapnia causes myocardial ischemia in normal healthy subjects seems even less likely. The Rutherford *et al.,* (2005) study is the only existing evidence that hypocapnia causes significant reductions in T wave amplitude. The present investigation suggests that these changes were not of ischemic origin and also questions the accuracy of the equipment used in the previous study to measure the ECG.

Changes in female sensitivity to CO_2

In women, the sensitivity of the respiratory system to changes in CO_2 alters depending on the stage of the menstrual cycle (Dutton *et al.*, 1989). The ventilatory responsiveness to changes in $PACO_2$ was found to significantly increase by 2% between the follicular phase and luteal phase and significantly decrease by 5% between the luteal phase and menstrual phase. Therefore, CO_2 sensitivity is significantly higher during the luteal phase of the menstrual cycle compared with the menstrual and follicular phases. This increase in CO_2 sensitivity is thought to be related to an increase in progesterone, which is elevated during the luteal phase (Dutton *et al.*, 1989). In the present investigation, this effect was not taken into account and therefore represents a potential limitation to the experiments conducted. It is not clear to whether changes in CO_2 sensitivity throughout the menstrual cycle affect the coronary vasoconstriction seen during hypocapnia.

If these experiments were repeated, it would advisable to control this variable by only studying female subjects during one phase of the menstrual cycle. It seems most appropriate to conduct experiments during the luteal phase of the menstrual cycle, at which point subjects would be most sensitive to changes in CO_2.

Modes of echocardiography

Another limitation of this thesis investigation was in the echocardiographic measurements used to assess heart function. Three modes of echocardiography were utilised; two-dimensional M-mode, Doppler blood flow and tissue Doppler analysis. The standard Doppler echocardiography used here assessed global left ventricular function by measuring the velocity of blood flow through the mitral valve and into the left ventricle during diastole.

Myocardial ischemia can cause diastolic dysfunction which would be reflected by a decrease in the velocity of blood flow into the left ventricle (Nishimura & Tajik, 1997). This measure of heart function is limited because it is insensitive to small changes in diastolic function (as might have occurred during hypocapnia). Therefore, only a large change in heart function would have been detected using this technique in the present investigation.

In addition to Doppler echocardiography, measures of regional wall motion were made using directed two-dimensional M-mode echocardiography and tissue Doppler analysis of the lateral wall and septum. Whilst these methods can accurately detect small changes in wall motion in the region of the myocardium being imaged, this method of grouping entire walls into one is not very specific and will result in large areas of the left ventricle being ignored.

If this experiment were to be repeated, it would be advantageous to take a different approach. Instead of using 3 different modes of echocardiography, it would be more beneficial to focus on small changes in heart function using detailed tissue Doppler analysis as described by Cerqueira *et al.,* (2002). Using this method, the left ventricle is divided into 17 segments across three levels (Base, mid-cavity and apex) split into anterior, septal, inferior, and lateral segments on each level. Each segment is imaged individually resulting in a more detailed analysis of left ventricular function.

Additional modes of echocardiography which could be used to assess left ventricular function include qualitative echocardiography and two-dimensional strain imaging. Qualitative echocardiography relies on the observer to classify myocardial wall segments according to their motion at rest and during myocardial stress. Segments are labelled as non-kinetic,

moderately kinetic or hyper kinetic. A difference between motion classifications would indicate a potential change in heart function. Two-dimensional strain imaging is advantageous because it is not dependant on the angle of the echocardiography transducer as in Doppler measurements. It relies on estimation of the vectorial velocities of every pixel within an echocardiographic image. These are used to calculate an angle-independant velocity of each wall segment over a specified time period (Citro *et al.*, 2008)

Hypocapnic 'hypoxia'

The third study of this thesis investigation attempted to accentuate the effects of hypocapnia in 21% inspired O_2 by hyperventilating subjects in 15% inspired O_2. No differences were found between ECG waveforms recorded in both conditions. A possible limitation to this study was that by mechanically hyperventilating participants, the effectiveness of breathing 15% inspired O_2 may have been diminished. When subjects inhaled 15% O_2 spontaneously, O_2 saturation measured using pulse oximetry was found to decrease to 93 ± 1%. When subjects inhaled 15% O_2 during mechanical hyperventilation, O_2 saturation remained at 100 ± 0%. It appears that during mechanical hyperventilation, the augmented breathing frequency and tidal volume increased the amount of O_2 being exchanged in the lung, therefore compensating for the lower proportion of O_2 in the air. O_2 delivery to the myocardium during hypocapnic hypoxia was consequently no different to O_2 delivery during hypocapnia in normal room air.

In order to achieve the desired reduction in O_2 saturation, it would be necessary to reduce inspired O_2 further, to a level which would not normally be considered safe in normal resting conditions. Using the methodology of the present investigation, it would be difficult to predict

the level of inspired O_2 needed to achieve this prior to experimentation. Therefore, if this experiment was to be repeated, a different method would be required.

An alternative approach would be to sample respired gas in each subject and measure end-tidal O_2 ($PetO_2$) instantaneously. This information could then be used in a simple feedback system to adjust inspired O_2 in each individual in order to achieve a predefined level of $PetO_2$. By titrating O_2 in this way, it allows the subject's $PetO_2$ to be accurately controlled regardless of whether they are hyperventilating or breathing normally. This technique has been successfully utilised by Balanos *et al.,* (2003), Talbot *et al.,* (2008) and Dorrington *et al.,* (2010) to study the effects of varying levels of $PetO_2$ and $PetCO_2$ on pulmonary vascular resistance. In these experiments, inspired O_2 is automatically adjusted by a computer system to achieve a predefined $PetO_2$. The same technique was used to alter $PetCO_2$ and induce hypercapnia. In the present investigation, mechanical hyperventilation would still be of use if this technique were to be utilised because it is still the most efficient method of reducing $PetCO_2$ in resting subjects.

Small patient population

An obvious limitation to final pilot study was the lack of patients studied. This made it difficult to draw conclusions as to the effects of hypocapnia on the ECG of patients with coronary artery disease. The primary reason for this small population of patients was the difficulty in gaining access to this specialist group of patients chosen for study. Whilst the prevalence of patients suffering from coronary artery disease is common, it is less common to find patients with established coronary artery disease who are not taking coronary vasodilator medications, haven't undergone coronary bypass surgery and are not suffering from any co-

morbidities (such as asthma, epilepsy, diabetes, renal failure or morbid obesity). Thus, from the beginning of the study the pool of eligible patients to recruit from was limited.

In addition, the number of eligible patients who declined participate in the experiment was higher than anticipated and on five occasions, patients willing to participate were denied permission to by their GP. Although reasons for non-participation were not required, most subjects stated a wish not to jeopardise their health any further by participating in an experiment which was designed to provoke a stress on the heart. When coupled with the limited number of eligible patients for recruitment in first place, this lack of participation resulted in a disappointingly low number patients examined over the study period.

If this pilot study was to be continued, it may be worth investigating the effect of hypocapnia on the ECG in patients who present with chest pain and have undergone an initial assessment of their risk of coronary artery disease. Studying patients with a relatively high risk of having coronary artery disease, before they undergo non-invasive cardiac imaging or coronary angiography, would increase the number of patients eligible for study and decrease the proportion of those eligible who are taking conflicting coronary vasodilator medication. This strategy of subject recruitment would also allow a direct comparison of whether there are any fundamental differences between the ECGs recorded during hypocapnia in patients eligible for diagnostic investigation who have coronary artery disease and those who do not.

During future patient recruitment, only acknowledgement of a consenting patient's participation would be requested from their GP. Since NHS policy does not require the GP to consent to a patient's participation in an experimental study of this kind, it has been possible

to gain ethical approval to do this. The advantage of this alternation in recruitment procedure is that it takes the responsibility of the patient's welfare (during the study) away from their GP, making it more likely that they will not intervene and prevent participation in the study.

7.4. Future experiments

As stated previously (in section 7.2), based on the findings of this thesis investigation, it seems unlikely that hypocapnia induced by mechanical hyperventilation will provoke myocardial ischemia in patients with coronary artery disease. To confirm this, it might be worth studying additional patients with varying severities of coronary artery disease under hypocapnic conditions to see if ECG changes occur. If this was to be done, the limitations highlighted in section 7.3 should be addressed. This would mean testing all female patients in the luteal phase of their menstrual cycle and using detailed (17 segment) tissue Doppler analysis to measure heart function. Should hypocapnia, induced by mechanical hyperventilation, still not result in measurable changes in cardiac electrical activity or heart function, then the preliminary assumption that this technique does not induce myocardial ischemia would be confirmed and therefore would not require further investigation.

Alternatively, if significant ECG changes did occur and it was assumed that hypocapnia does cause myocardial ischemia in patients suffering from coronary artery disease, there are subsequent experiments which might be of interest. The investigation of electrocardiographic responses to hypocapnia in patients presenting with chain pain of unknown origin would be of use in determining whether this technique of mechanical hyperventilation has any use in the early diagnosis of coronary artery disease. This study could be incorporated into a larger randomised controlled trial comparing the technique of hypocapnia induced by mechanical

hyperventilation with a gold standard test for coronary disease (coronary angiography) to establish the sensitivity and specificity of this provocative test and whether it is cost effective to be used in a clinical setting.

Another study of interest would be to investigate whether inducing hypocapnia with mechanical hyperventilation can be utilised with other cardiac imaging techniques. When used in conjunction with myocardial perfusion scintigraphy (MPS) using single proton emission computed tomography (SPECT), pharmacological stress agents such as adenosine, dipyridamole and dobutamine have a higher sensitivity for coronary artery disease than when they are used with echocardiography (91% vs. 79%, 90% vs. 72%, 84% vs. 81% respectively) (Heijenbrok-Kal *et al.*, 2007). Future experiments would therefore investigate whether hypocapnia induced by mechanical hyperventilation is practical with MPS using SPECT or wall motion/perfusion magnetic resonance imaging, whether it improves the sensitivity and/or specificity for coronary artery disease and whether the techniques are cost effective when used in conjunction.

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Appendix

Patient details

Patient 1A

Age: 42

Ethnicity: White British

Allergies: Penicillin

Past medical history:

- Asthma (5 years ago)
- Old gastric ulcer
- Angina (not resting or night pain)

Cardiovascular risk:

- Not diabetic
- BP normal
- Cholesterol normal
- Ex-smoker

Family history of cardiovascular disease:

- Mother had a MI aged 69
- Father had two MIs aged 61

Current medications:

- Isosorbide mononitrate
- Lansoprazole
- Bisoprolol
- Simvastatin
- Aspirin
- **Recommended for surgery**: Awaiting coronary angioplasty

Patient 2A

Age: 72

Ethnicity: White British

Allergies: None

Past medical history:

- Transient ischemic attack (2 years previously)
- Pleural plaques (no lung disease)
- Angina (no night pain, occasional rest discomfort)

Cardiovascular risk:

- Not diabetic
- Hypertension
- Hypercholesterolaemia
- Ex-smoker

Family history of cardiovascular disease:

- None

Current medications:

- Isosorbide mononitrate 20mg o.d.
- Bisoprolol 1.25mg o.d.
- Simvastatin 40mg o.d.
- Aspirin 25mg o.d.
- Ramipril 2.5mg o.d.
- **Recommended for surgery**: Awaiting coronary bypass graft

Patient 1B

Age: 54

Ethnicity: South Asian

Allergies: None

Past medical history:

- Duodenal ulcer
- Previous right coronary artery stents
- Angina (not resting or night pain)

Cardiovascular risk:

- Not diabetic
- Normal BP
- Hypercholesterolaemia
- Current smoker

Family history of cardiovascular disease:

- None

Current medications:

- Atenolol 50mg o.d.
- Simvastatin 40mg o.d.
- Aspirin 75mg o.d.
- Ramipril 2.5mg o.d.
- Lansoprazole 15mg o.d.
- Fenofibrate 67mg o.d.
- Finasteride 5mg o.d.

Recommended for surgery: Awaiting coronary bypass graft

Patient 2B

Age: 74

Ethnicity: White British

Allergies: Shellfish

Past medical history:

- Angina (not resting or night pain)

Cardiovascular risk:

- Not diabetic
- BP normal
- Cholesterol normal
- Ex-smoker

Family history of cardiovascular disease:

- None

Current medications:

- Simvastatin 40mg o.d.
- Aspirin 75mg o.d.
- Nicorandil 10mg o.d.
- Ranitidine 300mg o.d.
- GTN spray

Recommended for surgery: Awaiting coronary bypass graft