AUTONOMIC DYSFUNCTION IN CYSTIC FIBROSIS

Thesis submitted in accordance with the requirements of the University of Liverpool for the degree of Doctor of Medicine

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February 2004

ACKNOWLEDGEMENTS

I would like to thank all of the following people without whom this would not have been possible: my supervisors Dr M Walshaw and Dr M Ledson, Dr D Groves for his help with spectral analysis of heart rate variability, Dr J Earis for providing the equipment for recording of bowel sounds and Dr A Brown for analysing the recordings and Mrs C Edwards for her assistance with uroflowmetry.

In addition, I am grateful to the numerous medical and nursing staff at the Cardiothoracic Centre in Liverpool who acted as control subjects, to the staff in the outpatients department and the Adult Cystic Fibrosis Unit for allowing me to use their facilities and finally to the many cystic fibrosis patients who agreed to participate in this study.

TABLE OF CONTENTS

Page

Abstract
Autonomic Dysfunction in Cystic Fibrosis

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Autonomic dysfunction complicates chronic diseases such as diabetes mellitus, chronic liver and renal disease, malignancy and nutritional disorders. Preliminary work suggests that it may also exist in cystic fibrosis (CF). This study investigated autonomic dysfunction in adult CF patients across several body systems (cardiovascular, ophthalmic, gut and urinary) and compared it with disease severity.

Cardiovascular autonomic function was assessed using the traditional 5 Ewings tests and also power spectral analysis of heart rate variability. Ewings tests (10 controls and 38 patients) had variable reproducibility and abnormalities were observed in 0% to 50% of patients. Only the 30:15 ratio (heart rate response to standing) and the systolic blood pressure change on standing (an index of sympathetic function) differed significantly between controls and patients. In CF patients, there were significant relationships between worsening pulmonary function and the E:I ratio (a measure of the heart rate response to deep breathing) and 30:15 ratio. Power spectral analysis (38 controls and 49 patients) was reproducible and between 0% and 30.6% of patients had abnormalities. All spectral analysis parameters showed significant relationships with pulmonary function. Furthermore, the subgroup of patients colonised with *Burkholderia cepacia* had worse autonomic function in comparison to those colonised with *Pseudomonas aeruginosa*, which Ewing's tests could not detect.

Measuring the ratio of pupil to iris diameter (pupil diameter percent, PD%) in 14 controls and 35 patients assessed ophthalmic sympathetic function. There was no difference between these 2 groups, nor were there any correlations between PD% and markers of disease severity in the CF patients.

The recording and analysis of bowel sounds examined intestinal motility. Spectral analysis (18 controls and 16 patients) did not reveal any significant differences in mean power or median frequency between the 2 groups. However, there was a difference in median frequency between diabetic and non-diabetic CF patients. In the former, mean power correlated with use of intravenous antibiotics over the previous 2 years. 'CoolEdit' analysis of bowel sound frequency (16 controls and 17 patients) showed no difference between the groups, but CF patients with a history of constipation had significantly more bowel sounds than the remaining patients. There were no correlations with markers of disease severity.

Uroflowmetry and bladder ultrasound quantified maximum and average urine flow rates, voided volume and residual urine volume in 5 female patients. Using the Liverpool nomogram, 2 patients had average flow rates below the 10^{th} centile. The maximum flow rate also correlated negatively with glucose concentrations.

My study shows that autonomic dysfunction exists in CF. The clinical relevance of this should become clearer with the expected increase in survival in adult CF patients. Further work should also be done to look at the aetiology of these abnormalities. Nevertheless, when considering CF as a multisystem disease, autonomic dysfunction should be included.

CHAPTER 1: REVIEW OF THE LITERATURE

1:1 HISTORICAL ASPECTS

The first description of the autonomic nervous system (ANS) was made by Galen (born 130 AD) when he identified a nerve trunk lying along rib heads. He recognised it was connected to the spinal cord and believed the viscera received innervation from the brain through this. However, the mid-1880's saw the beginnings of the modern era of research into the ANS. In a series of experiments on dogs, Walter Gaskell attempted to anatomically define the major outflow of the ANS in terms of nerves arising from the 'cervico-cranial', thoracic and lumbosacral regions of the spinal cord (Gaskell, 1886). In addition, he described the structure and distribution of 'vasoconstrictor', 'accelerator' and 'augmentor' (sympathetic) nerves of the heart, as well as cardiac 'vasodilator' and 'inhibitory' (vagal) nerves. Furthermore, he stated that the action of the sympathetic cardiac nerves was to increase the strength and rate of contractions of cardiac muscle. Vagal nerve stimulation resulted in slowing of cardiac rhythm, decrease in muscle contraction and the 'lowering of excitability and conductivity'.

However, it was Langley (1898) who coined the term 'autonomic nervous system' to describe the 'sympathetic system and the allied nervous system of the cranial and sacral nerves, and for the local nervous system of the gut'. As a result of experiments on cats, Langley also showed that preganglionic fibres of the cranial autonomic system could make connections with sympathetic nerve cells and in doing so change their function. He concluded that there was no fundamental difference between preganglionic fibres and that 'the function of any autonomic nerve fibre depends not so much upon its inherent properties as upon the nerve cells with which it has the oppurtunity of becoming connected in the process of development'.

Loewi (1921) discovered that the parasympathetic system affected smooth muscle by means of a substance he called 'vagusstoff' (acetylcholine). In 1930, Walter Cannon performed experiments on a cat with heart denervated and spinal cord severed in the mid-thoracic region to permit painless operations on the hind part of the animal (Cannon and Bacq, 1931). Stimulation of the sympathetic supply to the smooth muscle of the tail nerves and blood vessels of that region caused a gradual increase in blood pressure, heart rate and salivary secretion. Cannon concluded

that since the only tissue known to be affected by sympathetic stimulation at the base of the tail was smooth muscle, since the only connection between the hind part of the animal and responding denervated organs in the fore part was the bloodstream and since interference with this markedly depressed the above responses, a substance given off from the smooth muscle was carried by the blood to the denervated organs. This substance also arose from the smooth muscle of the intestine, bladder and uterus. Cannon called it 'sympathin'.

Use of retrograde tracer methods originally developed by La Vail and La Vail (1972) has allowed further definition of the ANS. La Vail and La Vail reported the uptake and retrograde transport of horseradish peroxidase by axons of two different neuronal populations in the central nervous system of the chick. Not only could this be potentially useful in defining anatomical pathways but it could also suggest a possible mechanism for regulatory reactions such as neuronal chromatolysis after axonal injury or growth regulation according to the size of the peripheral field.

Nerve pathways in the ANS could also be elucidated by means of antidromic stimulation of neurones (Lipski, 1981). This involves stimulation of the peripheral part of the axon, evoking an action potential which travels towards the cell body where it can be recorded intra- or extracellularly. It therefore provides a method to precisely trace long axonal projections of neurons and is often complimetary to traditional neuroanatomical techniques.

More recently, the application of microneurographic and direct microelectrode recordings has allowed investigators to gain insight into the electrophysiological characteristics of, for example, sympathetic nerve activity in man by studying muscle nerve and skin nerve sympathetic activity (Wallin and Fagius, 1986).

1:2 ANATOMICAL ORGANISATION of the AUTONOMIC NERVOUS SYSTEM

The ANS has two major divisions, sympathetic and parasympathetic but also a third; the enteric or local nervous system of the gut (Langley, 1898). However, prior to Langley's work, it was Gaskell (1886) who initially described the anatomy of what are now recognised as the sympathetic and parasympathetic nervous systems. Langley's work was based on the dog because of the close resemblance to the distribution of nerves in man.

Anatomy of the sympathetic nervous system

Sympathetic nerves originate in the spinal cord between segments T1 and L2 (the so-called thoracolumbar outflow), and pass from here first into the sympathetic chain, thence to the tissues and organs that are stimulated by the sympathetic nerves (Shields, 1993).

The cell bodies of the neurons that compose the sympathetic efferent system lie in the intermediolateral (IML) cell column of the spinal cord. The preganglionic fibres that exit via the ventral spinal root are small (2-5 um) myelinated fibres, the white rami communicantes. These fibres then pass into one of the paravertebral or lateral ganglia (as described by Langley), paired ganglia lying adjacent to the spine and extending from the cervical to the sacral segments. The course of the fibres can then be one of the following two:

1. Some fibres may synapse on neurons in paravertebral ganglia at their segment of exit, or pass up or down many segments before synapsing in one of the other paravertebral ganglia. These so-called second-order neurons pass back from the ganglion into the spinal nerves. Langley recognised that these were thin unmyelinated fibres, the grey rami communicantes. Many of these fibres innervate blood vessels, sweat glands and hair follicles, whereas others will form plexi that supply the thoracic, abdominal, and pelvic organs.

2. Other fibres pass through the paravertebral ganglia without synapsing and form splanchnic nerves which synapse in more peripheral prevertebral or collateral ganglia. Langley termed these fibres as the rami efferentes. Second-order neurons then send their postganglionic fibres into the hypogastric, splanchnic and mesenteric plexi which innervate glands, blood vessels, and smooth muscles of the abdominal and pelvic viscera. Langley further reported that these second-order neurons connected with ganglion cells (called terminal ganglia) located either in the tissue of or in the immediate vicinity of the target organs.

Anatomy of the parasympathetic nervous system

The cell bodies of the parasympathetic efferent system lie in the nuclei of cranial nerves III, VII, IX and X, and in the IML cell column of the sacral spinal cord (the craniosacral outflow). The parasympathetic, like the sympathetic system, has both pre- and postganglionic fibres. However, in general, the preganglionic fibres pass uninterrupted to the organ that is to be controlled. The postganglionic neurons are located in the wall of the organ; the axons spread out into the sustance of the organ. Langley showed that about 75% of all parasympathetic nerve fibres are in the tenth nerve, the vagus nerve, and pass to the entire thoracic and abdominal regions of the body . The vagus nerve supplies parasympathetic nerves to the heart, the lungs, the oesophagus, the stomach, the small intestine, the proximal half of the colon, the liver, the gallbladder, the pancreas and the upper portions of the ureters.

Langley also indicated that the sacral parasympathetic fibres congregate in the form of the *nervi erigentes* which pass to the hypogastric plexus from where they distribute their peripheral fibres to the descending colon, rectum, bladder,

Higher centres

The supraspinal integration of ANS function is accomplished by a complex interaction of many brainstem, mesencephalic and cortical areas, including the nucleus of the tractus solitarius (NTS), nucleus ambiguus, dorsal motor nucleus of the vagus, medullary reticular formation, hypothalamus, limbic system and primary sensory and motor cortex. This complex arrangement of afferent and efferent interconnections has been delineated in a series of animal experiments. For example, Hopkins and Holstege (1978) studied amygdalotegmental projections in cats after injections of horseradish peroxidase and 3H-leucine and showed that pathways in this region played an important role in the integration of somatic and autonomic responses. In addition, Ricardo and Koh (1978) found anatomical evidence of direct projections from the NTS to the hypothalamus, amygdala and other forebrain structures in the rat with the aid of anterograde autoradiographic and retrograde horseradish peroxidase tracer techniques.

The neurotransmitter for all preganglionic fibres, both sympathetic and parasympathetic, is acetylcholine. This is also the neural transmitter for the postganglionic parasympathetic fibres. The postganglionic sympathetic transmitter is noradrenaline except for postganglionic fibres innervating the sweat glands which are cholinergic (Shields, 1993).

Acetylcholine receptors are divided into muscarinic and nicotinic subtypes (Parkinson, 1990*a*), whilst adrenergic receptors are composed of α and β subtypes (Parkinson, 1990*b*). α receptors are further divided into $\alpha 1$ and $\alpha 2$, which when activated result in an increased or decreased synthesis of cAMP respectively. The β receptors consist of $\beta 1$ and $\beta 2$ types. $\beta 1$ receptors generate positive inotropic and chronotropic effects on the heart. $\beta 2$ receptors are responsible for smooth muscle relaxation in eg bronchi and blood vessels to skeletal muscle.

1:3 FUNCTION of the AUTONOMIC NERVOUS SYSTEM

The autonomic nervous system is largely responsible for the regulation of visceral functions and the maintenance of homeostasis of the internal environment. It regulates visceral function primarily through its interaction with the endocrine system and via autonomic reflexes. These reflexes are capable of responding very quickly to alterations in the internal environment and can rapidly return the system to its homeostatic baseline (Shields, 1993).

Many target organs receive both sympathetic and parasympathetic innervation eg lungs, gastrointestinal tract and bladder, but there are notable exceptions; sweat glands, piloerector muscles, arterioles which receive only sympathetic innervation. In many target organs, the sympathetic and parasympathetic systems have antagonistic functions, but this is not uniformly true, particularly in those organs that receive only sympathetic innervation and in other organs, such as the lacrimal, parotid and submandibular glands which are stimulated to secretion by both the parasympathetic and sympathetic systems. However, in these glands, the parasympathetic system produces a more potent secretory effect (Shields, 1993).

The enteric nervous system also has several functions. It controls motility (Costa and Brookes, 1994), exocrine secretions (Li and Owyang, 1994), microcirculation of the gastro-intestinal tract (Suprenant, 1994) and regulates immune and inflammatory processes (Stead, 1992).

1:4 CLASSIFICATION of AUTONOMIC NEUROPATHY

The aetiology of autonomic failure can be primary or secondary.

Primary causes

Pure autonomic failure

This is not associated with other neurological disorders and was formerly called idiopathic orthostatic hypotension (Bradbury and Eggleston, 1925), but neurological disturbances of bladder, sexual function and sweating are also included.

Autonomic failure with multiple system atrophy

This was first described by Shy and Drager (1960); 'the full syndrome comprises orthostatic hypotension, urinary and rectal incontinence, loss of sweating, iris atrophy, external ocular palsies, rigidity, tremor, loss of associated movements, impotence, atonic bladder, fasciculations, wasting of distal muscle, evidence of a neuropathic lesion in the electromyogram that suggests involvement of the anterior horn cells and the finding of a neuropathic lesion in the muscle biopsy. The date of onset is usually in the 5^{th} to 7^{th} decade of life'.

Autonomic failure with Parkinson's disease

First described by Fichefet et al in 1965, this is characterised by Parkinson's disease plus mild orthostatic hypotension.

Secondary causes

These are more common and include a wide variety of chronic conditions such as diabetes, chronic liver disease, chronic renal failure, Vitamin B12 deficiency, alcoholism and nutritional disorders, malignancy and infectious diseases such as human immunodeficiency virus infection.

Diabetes

Cardiovascular reflex abnormalities can be detected at or shortly after diabetes has been diagnosed. For example, Fraser et al (1977) observed a significant abnormality in the ECG RR interval variations in resting heart rate in 6 out of 10 (60%) of newly diagnosed male diabetics. Abnormalities may also be found in diabetics of longer duration without any symptoms of autonomic neuropathy; Murray et al (1975) reported a reduction in 22 out of 42 (52%) of young asymptomatic male diabetics.

Over a period of years, the symptoms can progress into the florid picture of diabetic autonomic neuropathy initially recognised by Jordan in 1936; postural hypotension, nocturnal diarrhoea, delayed gastric emptying, bladder symptoms, abnormal sweating, impotence and inability to recognise hypoglycaemia. Possible pathophysiological mechanisms are discussed later.

Chronic liver disease

Thuluvath and Triger (1989) examined autonomic function in 64 patients with biopsy-proven liver disease (alcoholic and non-alcoholic). Parasympathetic damage was found in 45% and 43% of patients with alcoholic and non-alcoholic disease respectively. Sympathetic damage occurred in 11% and 12% of the patients. More recently, Fleckenstein et al (1996) demonstrated that the severity of autonomic dysfunction was associated with increasing severity of liver disease; autonomic damage was found in 14.3% Child A, 31.3% Child B and 60% Child C patients. Furthermore, a case report by McDougall et al (2002) documented improvement in autonomic neuropathy in a 40 year-old man with hepatitis-related

cirrhosis within 9 months of a successive liver transplantation, indicating the mechanisms are more likely to be metabolic in nature (see below).

Chronic renal failure

Whilst work by Nies et al (1979) and Naik et al (1981) concluded that autonomic dysfunction alone (on the basis of Ewing's tests of autonomic function) was not a sufficient explanation for chronic hypotension in some haemodialysis patients, more recent work by Kurata et al (2000), using the more sensitive 24 hour heart rate variability, plasma catecholamine level measurements and MIBG scanning, demonstrated cardiac autonomic neuropathy in patients with chronic renal failure on haemodialysis.

Vitamin B12 deficiency

Nerve conduction studies and sural nerve biopsies on patients with B12 deficiency have shown axonal degeneration (McCombe and McLeod, 1984) which can be arrested by replacement therapy, but residual neurological abnormalities, most commonly postural hypotension, may persist.

Alcoholism and nutritional disorders

Clinical manifestations of sympathetic autonomic dysfunction, such as postural hypotension, are unusual in uncomplicated alcoholic neuropathy (Low et al, 1975). However, Duncan et al (1980) demonstrated impaired heart rate responses to the Valsalva manoeuvre, deep breathing, change in posture and atropine in chronic alcoholics. Furthermore, histological studies have shown a reduction in the density of myelinated fibres in the distal vagus (Guo et al, 1987).

Malignancy

The Lambert-Eaton myasthenic syndrome (often associated with small cell carcinoma of the lung) is an autoimmune disorder in which IgG antibodies to voltage-gated calcium channels impair the release of acetylcholine from peripheral cholinergic nerve terminals (Kim and Neher, 1988). Autonomic dysfunction is well documented in this condition, manifestations including dry mouth, impaired lacrimation and sweating, impotence, constipation and orthostatic hypotension (Khurana et al, 1988).

HIV infection

Freeman et al (1990) studied patients infected with human immunodeficiency virus (AIDS plus AIDS-related complex) and controls, and observed a trend of declining autonomic function from controls to AIDS based on Ewing's tests. Thus autonomic dysfunction occurs with greater severity in AIDS patients; however, it may be present in the early stage of HIV infection and appears to progress during the illness. The mechanism is not known.

1:5 TESTS of AUTONOMIC FUNCTION

Autonomic dysfunction is a well documented complication of many chronic diseases, presenting with symptoms such as orthostatic hypotension, gastroparesis, impotence, bladder and bowel dysfunction (Ewing and Clarke, 1986).

Autonomic neuropathy is also clinically relevant in more acute situations. Faerman et al (1977) studied the autonomic nerve fibres to cardiac muscle in diabetic patients with painless myocardial infarction. In all cases, the fibres showed lesions typical of diabetic neuropathy; beaded thickening, fragmentation and dimunition in numbers of nerve fibres, unlike in those patients with painful infarction. The authors concluded that the absence of pain in diabetics with infarction could be due to a lesion of the afferent autonomic nerve fibres which conduct pain. Page and Watkins (1978) studied 8 diabetic patients who suffered cardiac arrests (and survived); all had evidence of autonomic neuropathy. This may be related to lengthening of the QT interval as reported by Ewing et al (1991), who recorded QT intervals twice at between 2 and 6 year intervals in 32 male diabetics with varying degrees of autonomic dysfunction. The QT interval had lengthened significantly at the second visit, which was unrelated to age or time between recordings, but which did correspond to changes in autonomic function. In addition, of 71 male diabetics under 60 years of age followed for 3 years, 13 had died, 8 unexpectedly, and of those with autonomic neuropathy, QT intervals were significantly longer in those who had died.

Patients with autonomic neuropathy also present an increased anaesthetic risk; Burgos et al (1989) found greater declines in heart rate and blood pressure during induction of anaesthesia in diabetics as compared to controls. 35% of the diabetics required intraoperative vaopressors compared with only 5% of controls. Furthermore, those who needed vasopressors had significantly greater impairment of autonomic test results.

These studies all emphasise the need for accurate, sensitive and specific tests of autonomic function which will now be discussed in detail.

1:5:1 CARDIOVASCULAR SYSTEM

Sympathetic and parasympathetic fibres innervate the atria, ventricles, coronary arteries and resistance vessels of the peripheral circulation (Ravits, 1997). Sympathetic activity increases heart rate and myocardial contractility, dilates the coronary vessels and constricts the resistance vessels; parasympathetic activity does the converse, except that it has little effect on the peripheral vasculature. Afferent activity originates in arterial baroreceptors located in the carotid sinus, aortic arch and various thoracic arteries, cardiac mechanoreceptors and pulmonary stretch receptors. Regulation is by a negative feedback system. This is best illustrated by the work of Mancia et al (1977) who investigated the baroreceptor reflex using the variable pressure neck chamber device previously described in 1975 by Eckberg et al; in brief, the neck is enclosed in a tight rigid collar extending from the shoulders to a plane intersecting the lower part of the face and skull. Pneumatic pressure in the collar can be reduced in a graded fashion below atmospheric pressure, thereby producing an increase in transmural pressure across the carotid sinuses and an increase in carotid sinus barorecptor activity. Mancia et al (1977) showed that increased baroreceptor stimulation resulted in a sustained fall in arterial pressure and a bradycardia. Conversely, decreased receptor stimulation had the opposite effects.

The assessment of cardiovascular autonomic function has been based on tests looking at heart rate and blood pressure change whilst performing certain manoeuvres.

Tests of heart rate change

Wheeler and Watkins (1973) found a reduction or complete absence in beat-to-beat variation of heart rate in diabetics with autonomic neuropathy, unlike control subjects or diabetics without autonomic neuropathy. Furthermore, administration of atropine intravenously to a control subject abolished the beat-to-beat variation but propranolol had no effect, indicating the importance of vagal control.

The Valsalva manoeuvre, which requires a subject to produce a respiratory strain with consequent changes in blood pressure and heart rate, was studied by Bennett et al (1978); measurement of the ratio of the bradycardia to the tachycardia evoked by this in diabetic subjects obviated the need to perform more invasive or tedious investigations such as estimation of baroreceptor reflex activity or apnoeic face immersion. Once again, the heart rate response could be abolished by atropine but not propranolol.

The immediate heart rate response to standing upright was assessed by Ewing et al (1978) in 15 diabetics with autonomic neuropathy. Comparison of the RR interval at around the 15^{th} beat (maximal tachycardia) to the RR interval at the 30th beat after standing (maximal bradycardia) showed little difference. In a control group, the response was abolished with atropine but not propranolol showing that it is mediated through the vagus nerve.

Tests of blood pressure change

Low et al (1975) measured the blood pressure response to standing in 16 subjects with diabetic neuropathy. 7 of these (44%) had postural hypotension. Quantitative histological studies of the greater splanchnic nerves at autopsy showed demyelination and a significant reduction in fibre density in diabetics. Disordered blood pressure control correlated with these findings.

Finally, Morley et al (1977) assessed 70 adult diabetics and reported evidence of sympathetic dysfunction, in particular abnormal blood pressure response to isometric exercise, in 28% of the group.

TESTS OF CARDIOVASCULAR AUTONOMIC FUNCTION

In 1982, Ewing and Clarke proposed a battery of 5 tests to assess autonomic function of any aetiology. 3 of these are predominantly parasympathetic and 2 mainly sympathetic.

Tests of parasympathetic function

These are:

1. Heart rate variability with respiration (sinus arrhythmia)
2. Heart rate response to the Valsalva manoeuvre
3. Heart rate response to standing from the supine position

Tests of sympathetic function

These are:

1. The diastolic blood pressure response to isometric exercise
2. The systolic blood pressure response to standing from the supine position

Tests of parasympathetic function

1. Heart rate variability with respiration (sinus arrhythmia)

Complex reflexes are responsible for the normal beat-to-beat variation in heart rate during quiet and deep breathing. Respiration is the most potent stimulus; changes in the depth and variation alter this effect. Pulmonary stretch receptors are the principal afferents for this reflex, Changes in vasomotor tone are also important influences (Wheeler and Watkins, 1973).

Disadvantages of the test

(a) Sinus arrhythmia is influenced by several factors

- **Age**

Wieling et al (1982) examined the heart rate changes induced by forced breathing in 133 healthy subjects aged 10 to 65 years. The lower limit of normal decreased from 22 to 11 beats per minute with increasing age. Similarly, O'Brien et al (1986) showed a negative correlation with age in 310 healthy subjects aged 18 to 85 years.

- **Resting heart rate**

Resting heart rate is another important factor. This was demonstrated during deep breathing in 75 normal subjects by van Dijk et al (1991) who therefore suggested that corrections for resting heart rate should be made when analysing results.

- **Variation in protocol**

Ewing et al (1981*a*) showed that recording the heart rate for 1 minute on an ECG while the subject breathed deeply at 6 breaths per minute was the best method of differentiating between diabetics with and without autonomic damage. However, some debate exists over whether the heart rate changes evoked by a single deep breath are greater than those evoked by repeated breaths. For example, Bennett et al (1978) claimed a single deep breath was a more potent stimulus for heart rate change. However, Espi et al (1982) demonstrated that the first deep breath

consistently produced the largest heart rate variation in only 29% of controls and 17% of diabetics and that the between-test variability was smaller with repeated deep breaths.

(b) Different methods of quantification exist

The maximum and minimum heart rates during each 10-second respiratory cycle can be measured and the mean of the differences gives the so-called maximum minus minimum heart rate (Hilsted and Jensen, 1979). Alternatively, these changes can be expressed as a ratio of the heart rate on expiration to that on inspiration, the 'E:I' ratio (Sundkvist et al, 1979).

Normal, borderline and abnormal values for maximum minus minimum heart rate are greater than or equal to 15 beats/minute, 11-14 beats/minute and less than or equal to 10 beats/minute respectively (Ewing and Clarke, 1982).

2. Heart rate response to the Valsalva manoeuvre

The Valsalva manoeuvre has four phases described in detail by Ravits (1997).

Phase I

This occurs at the onset of straining – there is a transient, few second-long increase in blood pressure due to an increase in intra-thoracic pressure causing mechanical 'squeeze' of the great blood vessels.. There are no neurally mediated changes in heart rate.

Phase II

This occurs with continued straining. There are 2 sub-phases:

In early Phase II, the blood pressure decreases due to a reduction in cardiac output as a result of decrease in venous return. After about 4 seconds (late Phase II), blood pressure recovers towards baseline as a result of a sympathetically mediated increase in total peripheral resistance. Throughout Phase II, there is a steady increase in heart rate initially due to vagal withdrawal, then from increased sympathetic activity.

Phase III

This occurs with release of the strain. There is a transient few second long decrease in blood pressure caused by mechanical displacement of blood to the pulmonary vascular bed (which was previously under increased intrathoracic pressure). There is no heart rate change.

Phase IV

Phase IV occurs with continued release of the strain. The blood pressure slowly increases to above baseline and heart rate decreases below baseline levels. This is also known as the 'overshoot'. It occurs 15-20 seconds after release of the strain and can last for longer than a minute. The mechanism is increasing venous return, increasing stroke volume and increasing cardiac output which returns blood pressure back towards baseline. The bradycardia is a vagal reflex in response to the rise in blood pressure.

The Valsalva ratio is the ratio of the longest RR interval during Phase IV the shortest RR interval during Phase II.

Disadvantages of the test

(a) **The Valsalva ratio is influenced by several factors**

- **Age**

As for sinus arrhythmia, the Valsalva ratio also declines with age. This was indicated by O'Brien et al (1986) in a study of 310 healthy subjects aged 18 to 85 years and by Low et al (1990) in 155 healthy subjects.

- **Expiratory pressure**

A choice of 40mmHg expiratory pressure is made because this seems to yield the most reproducible results; below 20mmHg is inadequate, above 60mmHg results in more variation as this is more difficult to maintain for the required 15 seconds (Korner et al, 1976).

- **Patient cooperation**

The Valsalva manoeuvre requires patient cooperation such that patients with weak expiratory facial or oropharyngeal muscles may be unable to participate. Furthermore, it should be avoided in patients with proliferative retinopathy because of the risk of retinal haemorrhage (Ewing and Clarke, 1982).

(b) **Only the compensatory change is recorded**

Without invasive beat-to-beat blood pressure recordings, only the compensatory heart rate change is being recorded instead of the true stimulus ie blood pressure. This can lead to occasional spurious results. For example, exaggerated drops of blood pressure during early Phase II from sympathetic failure can produce an exaggerated tachycardia which can normalise the ratio to the otherwise abnormal bradycardia. In addition, patients with parasympathetic failure who have preserved cardiac sympathetic function may have a tachycardia during the manoeuvre without subsequent bradycardia, thereby producing a normal ratio again (Low et al, 1975).

Normal, borderline and abnormal values for the ratio are greater than or equal to 1.21, 1.11-1.2 and less than or equal to 1.1 respectively (Ewing and Clarke, 1982).

3. Heart rate response to standing

The haemodynamic response to standing upright from the supine position involves closely linked changes in heart rate and blood pressure both of which will be described here.

On standing there is an immediate transient increase in blood pressure resulting from an increase in cardiac output (due to squeezing of capacitance vessels by postural muscles displacing blood back to the heart) and increase in peripheral resistance (due to squeezing of resistance vessels by postural muscles). These changes stimulate the baroreceptors with a subsequent decrease in sympathetic outflow and total peripheral resistance by up to 40% - blood pressure therefore drops. This lasts 6-8 seconds.

There is also an immediate increase in heart rate, maximal at 12-15 seconds. This is an exercise reflex which withdraws parasympathetic tone. Further baroreflex-mediated changes enhance sympathetic tone and the initial fall in blood pressure is corrected. Following this, a slowing of the heart rate occurs, maximal at 20-25 seconds; this is a vagal reflex due to increase in blood pressure (Ewing et al, 1978).

The heart rate response is quantified by the 30:15 ratio ie the ratio of the RR interval at about the thirtieth beat after standing to that around the 15^{th} beat after standing.

Disadvantages of the test

(a) The heart rate response to standing is influenced by age

Heart rate changes induced by standing decline with age as reported in 133 healthy subjects aged 10 to 65 years (Wieling et al, 1982) and in 310 subjects aged 18 to 85 years (O'Brien et al, 1986).

(b) **There is variation in methods of quantification**

There are investigators who believe that the initial heart rate response to standing should be quantified by the maximum RR interval after standing divided by the minimum RR interval after standing rather than the 30:15 ratio as there is slight variation in normal subjects about the points of shortest and longest RR intervals on standing (Ryder and Hardisty, 1990). Indeed, O'Brien et al (1986) found that in 294 normal subjects, 43% had a 30:15 ratio less than 1. However, there is a misconception in O'Brien's method where the count of beats is taken from the time the subject is actually standing up whereas RR interval change occurs immediately the subject starts to stand up (Ewing, 1990).

Normal, borderline and abnormal values for the 30:15 ratio (up to the age of 45 years) are greater than or equal to 1.04, 1.01-1.03 and less than or equal to 1 respectively (Ewing and Clarke,1982).

Tests of sympathetic function

1. Diastolic blood pressure response to isometric exercise

Sustained muscle contraction causes blood pressure and heart rate to increase, due to withdrawal of parasympathetic activity and increase in sympathetic activity and cardiac output (Ewing and Clarke, 1982). The test requires the patient to apply and maintain grip at 30% of the maximum for up to 5 minutes..

Disadvantages of the test

(a) The rise in diastolic pressure depends on resting blood pressure

The rise is independent of age (Goldstraw and Warren, 1985) but in a study of 75 normal subjects aged 8 to 96 years, van Dijk et al (1991) demonstrated a relationship with resting blood pressure.

(b) Limited reproducibility

The test has poor reproducibility; Piha et al (1991) found that coefficients of variation were in excess of 25% (see below).

The diastolic pressure should rise by at least 16mmHg; 11-15mmHg rise is borderline and less than or equal to 10mmHg is abnormal (Ewing and Clarke, 1982)

2. Systolic blood pressure response to standing

The haemodynamic response to standing has previously been described.

Orthostatic hypotension is a reduction in systolic pressure of at least 30mmHg (Ewing and Clarke, 1982). The diagnosis is only tenable after medical conditions are excluded. These include dehydration, haemorrhage, medications such as α and β antagonists, metabolic dysfunction such as adrenal insufficiency, hypothyroidism and septic shock.

Disadvantages of the test

(a) The response is related to resting blood pressure

As for the isometric exercise test, van Dijk et al (1991) found a relationship with resting blood pressure but not with age.

(b) Limited reproducibility

Comi et al (1986) reported a mean standard deviation of 3.702 in 10 healthy controls compared to 0.183 and 0.074 for the Valsalva and 30:15 ratios respectively.

Normal, borderline and abnormal values for the fall in systolic blood pressure on standing are less than or equal to 10mmHg, 11-29mmHg and greater than or equal to 30mmHg respectively.

Classification of severity of autonomic damage

Ewing et al (1985) compared a single test of autonomic function (heart rate response to deep breathing) with the full battery in 360 diabetics and showed that one test alone did not distinguish the severity of autonomic damage; use of just one test presumes that autonomic neuropathy is 'all or nothing' and does not allow for a range of nerve damage from minimal to extensive (Ewing and Clarke, 1986).

Autonomic neuropathy can be classified, according to the severity of damage, into one of four groups (Ewing, 1992):

- **Normal** – all five tests normal, or one borderline
- **Early involvement** – one of the three heart rate tests abnormal
- **Definite involvement** – two or more of the heart rate tests abnormal
- **Severe involvement** – two or more of the heart rate tests abnormal plus one or both of the blood pressure tests abnormal.

An alternative to this classification of severity is to give each individual test a score of 0, 1, or 2, depending on whether it is normal, borderline or abnormal respectively. An overall autonomic test score of 0-10 can then be obtained.

Reproducibility of the cardiovascular tests

Knowledge about reproducibility is important as the tests are commonly used in follow-up studies of autonomic neuropathy. There are few well documented reports concerning this. Piha et al (1991) conducted a study of 10 healthy subjects looking at short term (within a day) and long term (over 2 years) reproducibility. The coefficients of variation for the Valsalva ratio were 7% and 8% respectively. Values of 7% and 11% were obtained for the 30:15 ratio whilst the E:I ratio (an index of heart rate response to deep breathing) had values of 4% and 6%. However, the reproducibility of diastolic blood pressure rise with isometric exercise was much poorer; 34% and 24% respectively. Similarly, Comi et al (1986) demonstrated mean standard deviations of 6.019 and 3.702 for the sustained handgrip test and systolic blood pressure change on standing. By contrast, the standard deviations for the Valsalva and 30:15 ratios were 0.183 and 0.074.

A reproducible circadian rhythm of heart rate variability has also been observed in normal subjects by Huikuri et al (1990). This group performed 24 hour ambulatory ECG recordings while subjects undertook their usual daily activities. The mean intraindividual coefficient of variation for the 24 hour average heart rate variability between repeated recordings was 7%.

The sequence of abnormalities in autonomic neuropathy

There is evidence that parasympathetic damage precedes sympathetic dysfunction. Gluck et al (1979) carried out an assessment of 23 diabetic patients. Beat-to-beat variation in heart rate was abnormal in all 9 diabetics with an increased clinical score and in 9 out of 14 diabetics with a normal clinical score. The pressor response to handgrip was only slightly reduced in the diabetic group. Additional indices of adrenergic function such as the pressor response to a cold stimulus, plasma renin and catecholamine excretion rates did not differ significantly between controls and diabetics. Ewing et al (1981*b*) investigated heart rate changes in 61 diabetics with varying degrees of cardiovascular reflex abnormalities. Those with parasympathetic abnormalities alone had the highest heart rates, whilst those with both parasympathetic and sympathetic damage had slightly slower heart rates which were still faster than those with normal cardiovascular reflexes. 13 other subjects whose autonomic function changed from normal to abnormal showed a sequential increase in heart rate as cardiac parasympathetic damage developed, followed by a fall in heart rate as sympathetic dysfunction developed. A study of 543 diabetics (Ewing et al, 1985) reported that abnormalities of heart rate tests occurred in 40% whilst abnormal blood pressure tests occurred in less than 20%. Of 237 subjects whose tests were repeated at least 3 months apart, the results of 71% were unchanged, 26% deteriorated and only 3% improved. The worsening followed a sequential pattern with first heart rate and later additional blood pressure abnormalities.

More recently, Quadri et al (1993) found that the impairment of a comprehensive evaluation score in 90 diabetics over a 5 year period (from 2.5 ± 1.7 to 3 ± 1.5, $p<0.05$) also confirmed a deterioration in autonomic. Finally, Toyry et al (1996) examined 133 diabetics and 144 controls at baseline, 5 years and 10 years. The frequency of parasympathetic neuropathy (patients versus controls) was 4.9% versus 2.2% ($p=0.224$) at baseline, 19.6% versus 8.5% ($p=0.017$) at 5 years and 65% versus 28% ($p<0.001$) at 10 years. The frequency of sympathetic dysfunction was 6.8% versus 5.6% ($p=0.709$) at 5 years and 24.4% versus 9.0% ($p=0.003$) at 10 years.

The mechanism for earlier parasympathetic damage is not known; it may be that cardiac vagal fibres are damaged earlier than sympathetic fibres as they are longer and therefore more liable to damage.

Problems with Ewing's tests

Although historically the assessment of cardiovascular autonomic function has been based on Ewing's tests, it is apparent that these have several disadvantages.

1. They have been criticised as being insensitive to early changes in autonomic function, particularly sympathetic nervous system dysfunction (Low et al, 1986).
2. The tests of sympathetic function have poor reproducibility with coefficients of variation in excess of 30% (Piha et al, 1991) and therefore are much less reliable.
3. They require active patient participation (Lishner et al, 1987; Freeman et al, 1991), which is difficult to standardise.
4. Conventional scoring systems rely on being able to perform all 5 tests (Ewing, 1992). However, the Valsalva manoeuvre is difficult to perform particularly in the presence of chronic lung disease.

The technique of power spectral analysis may answer some of these criticisms.

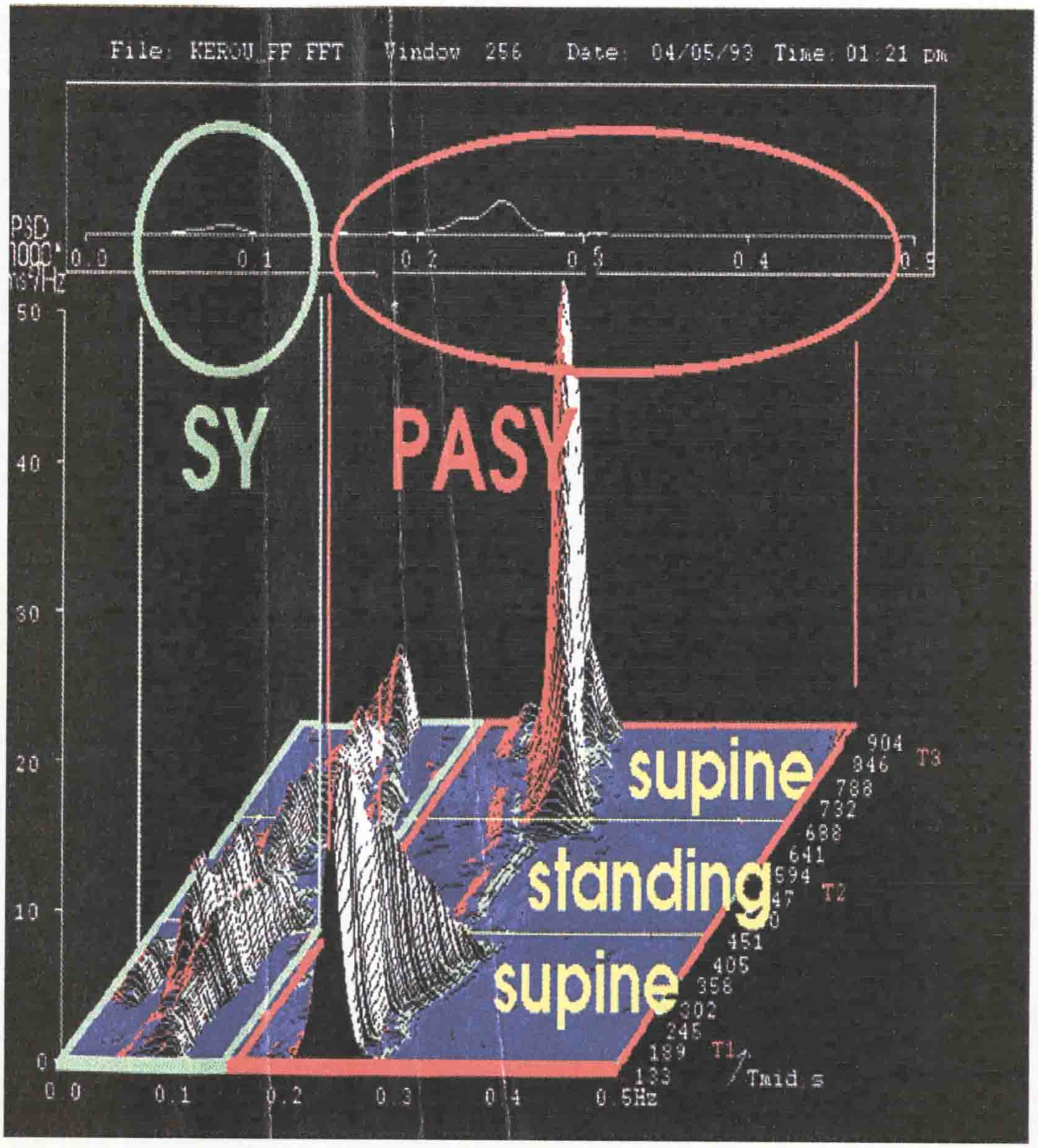
Power spectral analysis

Any biological rhythm such as heart rate, or an RR time interval series, can be broken down into a series of sine waves of different amplitudes and frequencies (Freeman et al, 1991). Spectral analysis, using fast Fourier transformation, separates the RR interval time series into groups of identical discrete frequencies (Ewing, 1992). The power spectrum displays the amplitude squared of these sine waves against frequency. Power spectral analysis may thus provide a useful non-invasive technique for analysing the autonomic mechanisms that control heart rate. Spectral analysis of the resting heart rate commonly produces several prominent peaks (see Figure). These peaks can be further quantified by measuring the area under each. The peak found at the highest frequency (greater than 0.15 Hz) reflects oscillations of heart rate that occur with respiration (the respiratory sinus arrhythmia). This is a reflection of parasympathetic (vagal) activity and is abolished by atropine (Pomeranz et al, 1985).

Oscillations in heart rate at frequencies between 0.05 Hz and 0.15 Hz (low frequency) represents baroreceptor feedback activity, whilst the peak at less than 0.05 Hz (very low frequency) reflects fluctuations in vasomotor tone associated with thermoregulation. These provide an index of sympathetic activity (Akselrod et al, 1985) and are diminished by propranolol.

Thus spectral analysis provides a single test of autonomic function, measuring both parasympathetic and sympathetic divisions of the autonomic nervous system, requiring minimal patient co-operation (Ziegler et al, 1992).

Figure: Power spectrum of heart rate variation



The power spectrum of heart rate variation has previously been characterised in several conditions. Lishner et al (1987) evaluated diabetic autonomic neuropathy in 23 subjects by computerised spectral analysis of RR interval variations and found a statistically significant decline in the power spectrum in all frequency ranges (most notably at the respiratory peak) when compared to controls. This difference persisted when the comparison was made within age groups below and above 65 years.

Spontaneous heart rate fluctuations have also been studied in patients with severe chronic congestive cardiac failure (Saul et al, 1988). This group reported a significantly decreased power in all frequency bands compared to controls. There was also a weakly significant positive correlation between absolute power in the 0.04 to 0.07Hz band and cardiac index ($r=0.466$, $p<0.05$) and inverse correlation with the pulmonary capillary wedge pressure ($r=-0.545$, $p<0.01$).

Lazzeri et al (1997) investigated 24 hour heart rate variability in 12 non-alcoholic cirrhotic patients with ascites. Compared to controls, these patients showed a decrease in the low frequency component of the power spectrum. More recently, Coelho et al (2001) examined 24 hour heart rate variability in 22 cirrhotic patients (alcohol related, viral hepatitis and autoimmune aetiologies). A significant decrease in all spectral analysis parameters was found in this group when compared to controls.

Power spectral analysis has also been used to evaluate autonomic dysfunction in uraemic patients on haemodialysis. Vita et al, 1999 studied 30 patients on periodic bicarbonate haemodialysis. The power spectrum of heart rate variability was a good discriminator of low and high frequency bands in both patients with and without Ewing's tests-proven autonomic neuropathy. Furthermore, a significant reduction of low frequency values in supine uraemic patients without autonomic neuropathy appeared to demonstrate early sympathetic dysfunction which Ewing's tests were unable to detect.

Preliminary work has suggested that the ANS in cystic fibrosis (CF) becomes damaged with increasing disease severity; Tattersall et al (2001) reported strong correlations between total autonomic function, sympathetic and parasympathetic activity and spirometric indices in 26 adult CF patients.

1:5:2 GASTROINTESTINAL SYSTEM

The gastrointestinal tract is the organ system with the largest surface in the body, serving motor, secretory, storage and excretory functions. There are two main control systems: the intrinsic (enteric) nervous system consists of approximately 10^8 semiautonomous neurons located in the wall of the gastrointestinal tract, and provides specific programmes and reflexes for the control of gastrointestinal motility (eg the peristaltic reflex) and other functions, whilst the extrinsic (sympathetic and parasympathetic) nervous system modulates the intrinsic reflexes and integrates impulses from higher nerve centres and the gut (Bittinger et al, 1999).

Autonomic dysfunction of the gastrointestinal tract can be due to disturbances of the intrinsic or extrinsic systems. The sequelae of this have mainly been studied in diabetes mellitus. Feldman and Schiller (1983) studied the frequency of gastrointestinal symptoms in 136 diabetics and found that 76% suffered from at least one symptom with constipation being the most common in 60% of patients. However, diagnosis of autonomic dysfunction in the gastrointestinal tract is difficult for several reasons:

1. The symptoms of autonomic dysfunction are non-specific and in fact some patients are symptom-free.
2. With the exception of 2 tests (pancreatic polypeptide response to sham feeding or hypoglycaemia {= parasympathetic test} [Krarup, 1979] and superior mesenteric artery blood flow studies in response to stress {= sympathetic test} [Chaudhuri et al, 1992], there are no specific tests for the detection of autonomic neuropathy of the gastrointestinal tract. Diagnostic workup is therefore usually restricted to the investigation of sequelae of autonomic dysfunction eg disturbed motility.

Oesophagus

Autonomic dysfunction in the oesophagus can lead to dysphagia (due to disturbed motility) and/or heartburn (due to gastro-oesophageal reflux). This can be investigated by oesophageal manometry and 24 hour pH monitoring. Typical manometric findings are low contraction amplitudes, delayed propagation of contractions, an increased occurrence of simultaneous contractions and a low resting pressure of the lower oesophageal sphincter (Loo et al, 1985).

It is thought that abnormalities of vagal function contribute to the pathogenesis of reflux disease. This is supported by observations that the gastric secretory response to insulin-induced hypoglycaemia is impaired in some patients with reflux (Ogilvie et al, 1985) and that approximately 40% of patients with reflux disease have abnormal parasympathetic cardiovascular reflexes (Chakraborty et al, 1989). In addition, Cunningham et al (1991) found that in patients with gastro-oesophageal reflux disease, oesophageal transit was significantly slower in the presence of abnormal parasympathetic cardiovascular reflex tests.

Stomach

Delayed gastric emptying (causing nausea and vomiting, and due to vagal dysfunction) is a common manifestation of autonomic neuropathy. It can be found in up to 50% of diabetics (Horowitz et al, 1989). The gold standard for evaluation of gastric emptying is scintigraphy using the dual tracer method (Horowitz et al, 1985).

Small bowel

Autonomic dysfunction involving the small intestine can disturb fasting and post-prandial motility patterns.

Normally in the fasted state, the small bowel shows a characteristic motility pattern termed the migrating motor complex (MMC) which can be divided into 3 phases: Phase I is characterised by a period of almost complete motor inactivity. Phase II shows intermittent, predominantly incoordinated motor activity. Motor activity increases during Phase II and then turns into a very characteristic motility pattern, the Phase III of the MMC. During this period, the small bowel exhibits rhythmic contractions which migrate from the stomach towards the colon. After food intake the small bowel shows Phase II-like more uniform contractions at all levels (Bittinger et al, 1999). In patients with autonomic dysfunction these motility programmes are often disturbed with consequent pseudo-obstruction symptoms of nausea, vomiting, or diarrhoea (often due to bacterial overgrowth).

Small bowel manometry best evaluates motility. Typical manometric findings in autonomic dysfunction are aberrant propagation or even loss of Phase III of the MMC, a persistence of the fasting motility pattern after food ingestion and intensive uncoordinated 'bursts' of phasic pressure activity (Camilleri and Malagelada, 1984).

Colon

The typical complaint of patients with autonomic dysfunction involving the colon is constipation (Feldman and Schiller, 1983). The pathogenesis is not well understood, but it has been shown that, for example, diabetic patients with cardiovascular autonomic neuropathy have prolonged colonic transit times, in particular of the right colon, compared with diabetics without cardiovascular autonomic neuropathy (Werth et al, 1992). More important is the observation that diabetics with severe constipation show a complete loss of the normal post-prandial rise in colonic electrical and motor activity, the so-called gastrocolic reflex. This myoelectrical activity can be stimulated pharmacologically, therefore

a myogenic cause is unlikely and so it was concluded that autonomic neural dysfuntion is the main cause of this disturbed reflex (Battle et al, 1980).
Diagnostic procedures in the evaluation of colonic motility are colonic transit time studies with radio-opaque markers (Metcalf et al, 1987), and manometry (Bittinger et al, 1999).

It can be seen that the above investigations are complex and invasive. They also involve radiation exposure and transfer of the patient to a specialised unit. Therefore, a potentially simpler, non-invasive test will now be described.

The investigation of bowel sounds

Bowel sounds were first recorded by Cannon in 1905 as a means of studying the mechanical activity of the gastrointestinal tract. Using a Bowles stethoscope, a telephone receiver and a nerve muscle preparation connected to a smoked drum, he recorded bowel sounds with an amazing degree of accuracy.

Arnbjornsson (1986) looked at bowel sounds in 7 patients with clinical, radiological and surgical evidence of mechanical small bowel obstruction. Sounds were monitored for 60 minutes by 4 microphones placed on the abdominal wall, one in each quadrant. The most striking observations were the regular occurrence of clustered bowel sounds, defined as 3-10 regular sounds occurring one every 5 seconds, preceded and followed by at least one minute of silence, and also a high motility index (an indication of the motor activity of the intestine). Yoshino et al (1990) recorded bowel sounds for 15 minutes in each of 21 patients with mechanical obstruction using a microphone covered with foam rubber placed over the wall of the right lower abdomen in a sound proof room. Bowel sounds were classified into 3 types based on frequency ranges that related to subsequent clinical management of the patients. Type I sounds had lower and upper frequencies of 173Hz and 667Hz and patients in this category were diagnosed as having simple obstruction caused by postoperative adhesions; all were treated conservatively. Type II sounds had lower and upper frequencies of 309Hz and 878Hz. These patients had simple obstruction due to postoperative adhesions, carcinomatous peritonitis and Crohn's disease; 3 underwent surgery. Type III

sounds had lower and upper frequencies of 330Hz and 766Hz, but unlike with Type II sounds, there was an absence of high frequency sounds above 900Hz and the sounds were generally of lower amplitude. These patients had strangulating obstructions accompanied by a disturbance of intestinal circulation; all were treated surgically. Thus it appears that bowel sound analysis could provide objective assessment of the severity of obstruction and help determine treatment.

Sugrue and Redfern (1994) recorded bowel sounds for 10 minutes in 21 obstructed patients with a microphone secured to the right lower iliac fossa with self-adhesive tape. Bowel sounds were significantly longer compared to controls (37msec versus 20msec, $p<0.05$) and of greater amplitude (patients versus controls, 238mV versus 70mV, $p<0.05$).

There is, however, little information on how well bowel sound recordings correlate with manometric profiles (manometry is considered to be the gold standard test of intestinal motility). Cullen et al (1989) evaluated surface vibration analysis (SVA) against manometry in partially obstructed patients (SVA is a technique which uses an electromechanical transducer mounted externally on the abdominal wall). The manometric profile in the jejunum (proximal to the obstruction) was characterised by regular periodic bursts of hyperactivity and inactivity, and was accompanied by a synchronous SVA response of hyperactivity and quiescence. Thus the SVA profile was considered to representative of the activity of the proximal obstructed jejunum.

Meconium ileus equivalent/distal intestinal obstruction syndrome (DIOS) are also significant problems in cystic fibrosis. Hanly and Fitzgerald (1983) showed that of 53 patients attending an adult CF unit, 8 had experienced a total of 25 episodes of meconium ileus equivalent. Recurrent attacks occurred in 7 patients, of whom 4 had at least 4 separated well documented episodes. All patients responded to appropriate medical treatment including 3 who had previously undergone surgery elsewhere for meconium ileus equivalent. Rubinstein et al (1986) reported that of 168 CF patients, over 30% experienced at least one episode of constipation; meconium ileus equivalent developed in 9%. Patients younger than 5 years of age had a lower prevalence and those older than 30 years had a much higher prevalence of both conditions – those with prolonged histories of inadequately controlled steatorrhoea appeared to be at higher risk for the eventual development of meconium ileus equivalent. Furthermore, oro-caecal transit time, as assessed by

the lactulose/hydrogen breath test, has been reported to be prolonged in CF patients experiencing DIOS compared to those without the syndrome and normal controls (Dalzell and Heaf, 1990).

1:5:3 URINARY SYSTEM

The autonomic innervation to the bladder is complex, but essentially during bladder filling (urine storage) sympathetic activity results in detrusor muscle inhibition and internal sphincter contraction; parasympathetic outflow is inactive. During voiding, sympathetic outflow becomes inactive whilst parasympathetic activity causes detrusor muscle contraction (de Groat, 1992).

Bladder dysfunction has been recognised as a frequent complication of diseases affecting the autonomic nervous system such as multiple system atrophy, pure autonomic failure and diabetes. The best method of assessment utilises simple uroflowmetry studies and more complex urodynamic studies.

Multiple system atrophy (MSA)

Early urinary symptoms include urgency and frequency of micturition and reduced urine stream. However, as the strength of the detrusor contractions during voiding diminishes, the bladder fails to empty and large post-micturition residual urine volumes develop. Urodynamic studies have shown detrusor hyperreflexia in the early stages of the disease, but later as disease progresses and the bladder becomes atonic high residual volumes are seen. Voiding becomes intermittent, incomplete and is achieved mainly by abdominal straining (Betts and Fowler, 1992).

A recent study of MSA patients has reported that 96% had urinary symptoms and 74% had post-micturition residuals, and urinary dysfunction appeared to be more common and often an earlier manifestation than orthostatic hypotension (Sakakibara et al, 2000*a*).

Pure autonomic failure (PAF)

This is characterised by widespread autonomic failure without central nervous system involvement. Sakakibara et al (2000*b*), investigated 6 patients with PAF. All had urinary symptoms. Furthermore, urodynamic studies demonstrated post-micturition residuals in 2 and detrusor hyperreflexia in 4, indicating that micturitional disturbance is a common feature of PAF.

Diabetic cystopathy

Bladder dysfunction is a well recognised feature of diabetic autonomic neuropathy, although its exact prevalence is unknown as patients are often asymptomatic (Ewing and Clarke, 1986). Compared with control subjects, cystometrograms in these patients exhibit significant increases in bladder volume at first desire to void and maximal bladder capacity, a decrease in detrusor contractility and a larger mean residual volume of urine (Ueda et al, 1997).

The sequence of pathophysiological events that result in diabetic neurocystopathy indicate that there is involvement of the sensory afferent fibres causing reduced awareness of bladder filling, involvement of the parasympathetic fibres to the detrusor muscle decreasing the ability of the bladder to empty. In addition, the density of acetylcholinesterase positive staining nerves in the bladder wall is reduced compared with controls (Faerman et al, 1973).

The bladder neck, which is principally innervated by sympathetic fibres, is competent in most cases of diabetic cystopathy, suggesting that sympathetic denervation is probably a late phenomenon (Betts and Fowler, 1992).

1:5:4 OPHTHALMIC SYSTEM

Contraction of the circular smooth muscle fibres of the sphincter pupillae in response to parasympathetic activity constricts the pupil on exposure to light and near vision. Conversely sympathetic activity results in dilatation of the pupil in darkness and during arousal (Smith, 1992).

Pupil function has been most widely studied in diabetes mellitus. Four tests are available.

Tests of sympathetic function

Resting pupil darkness diameter

Re-dilatation time

Tests of parasympathetic function

Pupil cycle time

Light reflex latency

Resting pupil darkness diameter

In the human eye, light reflex latencies are in excess of 0.2s, and so conventional flash light photographs can be taken even in darkness before the pupil start to constrict. A convenient way of expressing pupil size is pupil darkness diameter percent (PD%). This is the ratio of the pupil to iris diameter as a percentage of the latter. Using flash light photographs Smith and Dewhurst (1986) demonstrated that a large proportion of diabetic patients had abnormally small pupils. The results were consistent with the standard television pupillometric technique and had the same repeatability. Similar findings were obtained in adolescents by Schwingshandl et al (1993) using infra-red computerised pupillometry and by Karavanaki et al (1994) using Polaroid pupillometry.

Redilatation time

This is the time to three-quarters dilatation following maximal constriction by a light reflex, and is increased in diabetic patients (Smith, 1992).

Pupil cycle time

Regular oscillations of the pupil are induced by focussing a narrow beam of light on the pupil margin using a slit lamp. The constricting pupil interrupts the light beam, removing the stimulus, thereby dilating the pupil enabling the light to restimulate the retina. Mean time is calculated from 100 cycles with a stopwatch (Smith, 1992). It is prolonged in diabetes and said to correlate well with cardiovascular autonomic neuropathy (Martyn and Ewing, 1986).

Light reflex latency

This can be measured from the response of the pupil to short-duration light stimuli in background darkness. It is said to be prolonged in diabetic patients more often than abnormal PD% (Lanting et al, 1990*a*).

Pharmacological pupil tests are a useful supplement to the above. They rely on the principle of denervation hypersensitivity whereby an organ, eg the eye, deprived of its innervation becomes more sensitive to the transmitter normally released from those nerves.

In sympathetic dysfunction the pupil is supersensitive to the mydriatic effect of topical 2% phenylephrine (Smith and Smith, 1983), whereas in parasympathetic dysfunction the pupil is supersensitive to the miotic effect of pilocarpine (Cahill, 2001).

Similar results have been demonstrated in patients with cystic fibrosis in response to phenylephrine and carbachol (Davis et al 1980).

1:5:5 RESPIRATORY SYSTEM

Autonomic dysfunction involving the respiratory tract results in abnormalties of bronchomotor tone, airways responsiveness, ventilatory response to exercise, respiratory drive and sleep-related breathing. These will now be discussed in more detail below.

Bronchomotor tone

Normally bronchial tone is sustained by the tonic cholinergic activation of smooth muscle fibres in the airways. Alterations in bronchomotor tone were initially investigated in diabetic autonomic neuropathy. The administration of the anticholinergic agent, ipratropium bromide, has been shown to produce a smaller increase in bronchial calibre in diabetics with autonomic dysfunction as compared to those without, suggesting that patients with autonomic neuropathy have reduced airway vagal tone (Douglas et al, 1981).

Airway responsiveness

Specific airway responsiveness is a functional characteristic of the airways whereby bronchoconstriction develops after exposure to stimuli such as cold air, methacholine and histamine.

Heaton et al (1984) studied 5 diabetics with severe symptomatic autonomic neuropathy. After bronchial provocation testing with cold air, these patients did not show a significant fall in specific airway conductance unlike those diabetics without autonomic neuropathy or controls. Subsequently, Tantucci et al (1988) performed a study looking at the effects of increasing doses of inhaled methacholine on airway calibre in 2 groups of diabetic patients with and without autonomic neuropathy. Bronchoconstriction was less in the former group. Methacholine acts directly on specific surface receptors in bronchial smooth muscle and so the reduced airway responsiveness was interpreted as the loss of reflex bronchoconstriction which results from stimulation of these receptors innervated by vagal fibres. Furthermore, Bertherat et al (1991) investigated the magnitude of the response to methacholine in 22 insulin-dependent diabetics. This

was markedly reduced in those patients with one or more abnormal results by cardiovascular assessment of autonomic control.

In contrast to these studies, Rhind et al (1987) reported an increased airway responsiveness to histamine in a group of diabetics with autonomic neuropathy and suggested that it could be due to denervation hypersensitivity, although the selection of subjects (most of whom were smokers) casts doubt over the validity of the results. This highlights the importance of subjects having no evidence of lung disease which would otherwise be a major confounding factor when investigating autonomic function in the respiratory system.

Exercise

Tantucci et al (1996) examined the ventilatory response to exercise in terms of breathing pattern and activity of respiratory centres in diabetics with autonomic neuropathy. The increase in minute ventilation with increasing CO_2 production during exercise was higher in these patients as compared to controls or diabetics without neuropathy, and was sustained by a higher respiratory rate (rather than tidal volume). There was also an increase in alveolar ventilation and neuromuscular output from the respiratory centres (measured as mouth pressure 0.1sec after the onset of an occluded inspiration) during exercise in the patients with autonomic neuropathy. However, no differences were observed between any of the groups at rest.

Respiratory drive

Since the activity of the respiratory centres is to a large extent regulated by arterial pH, pO2 and pCO2, the hypoxic and hypercapnic stimulations to respiratory drive have been used as tools to investigate the influence of diabetic autonomic neuropathy on the control of breathing. Consistent decreases in hypoxic drive have been found in diabetics with autonomic neuropathy, indicating a reduced peripheral chemosensitivity in these patients, as compared to controls or diabetics without neuropathy (Williams et al, 1984). This was ascribed to a damage of cholinergic fibres in the glossopharyngeal afferent nerve from the carotid bodies.

Tantucci et al (1996) split diabetic subjects into 2 groups, characterised by the clinical presence of either parasympathetic and sympathetic damage with postural hypotension (Group A), or predominantly parasympathetic damege without postural hypotension (Group B). The increased hypercapnic drive in Group A compared to Group B suggested an inhibitory effect exerted by the sympathetic nervous system on central chemosensitivity in these patients. Indeed, previous animal studies have indicated that stimulation of pulmonary sympathetic afferent fibres inhibits phrenic nerve discharge in anaesthetised dogs and monkeys (Kostreva et al, 1978).

Sleep-related breathing disorders

In a small study, Guilleminault et al (1981) reported obstructive sleep apnoea syndrome (OSAS) in 2 out of 4 diabetics with autonomic neuropathy, whilst another had central sleep apnoea. These patients also showed an absence of heart rate change during the episodes of apnoea. Ficker et al (1998) conducted a larger study of 23 diabetics with autonomic neuropathy and 25 without. All patients were evaluated for OSA (apnoea/hypopnoea index > 10) with full polysomnography. 6 out of 23 (26%) of patients with neuropathy had OSA but none of those without.

The pathophysiological mechanisms of OSA in autonomic neuropathy are unknown. However, Weiner et al (1982) assessed the effects of hypoxia and hypercapnoea on the activity of the cranial nerves supplying upper airway muscles in paralysed anaesthetised artficially ventilated dogs after vagotomy and found that there was a differential response between the cranial and phrenic nerves which could potentially affect the forces during inspiration and lead to obstruction of the upper airway.

1:5:6 THERMOREGULATORY SYSTEM

Thermoregulation is controlled by the sympathetic nervous system with the parasympathetic system playing a minor role.

Sympathetic sudomotor fibres innervate sweat glands. Sympathetic vasomotor fibres cause vasoconstriction of cutaneous vasculature (Ravits, 1997).

Sudomotor function depends on the integrity of the sympathetic neural pathways, the eccrine sweat glands and the skin.

Briefly, 4 tests are available: the thermoregulatory sweat test (TST), the quantitative sudomotor axon reflex test (Q-SART), the sweat imprint test and peripheral autonomic skin potential recordings.

Thermoregulatory sweat test

The TST evaluates the overall autonomic regulation of body temperature, specifically cooling, by providing a physiological stimulus, heat, and recording the body's ability to dissipate it by sweating (Fealey et al, 1989). Unfortunately, the test is time-consuming, messy and requires specialist equipment. Abnormalities have been reported in up to 70% of patients with distal small fibre neuropathy (Stewart et al, 1992).

Q-SART

Sweat gland function can be assessed by indirect chemical activation from axon reflexes using acetylcholine iontophoresis. The test is sensitive and reproducible. However it is time consuming, requires specialist equipment and is not widely available (Ravits, 1997). In diabetes the test is comparable to tests of vagal function with both being more sensitive in detecting autonomic neuropathy than either one alone (Low et al, 1986).

Sweat imprint test

This measures sweat output at a fixed time after a stimulus by visualising and quantifying sweat droplets secreted by sweat glands (Ravits, 1997). Unfortunately the test is time-consuming and requires specialised equipment.

Peripheral autonomic skin surface recordings

A peripheral nerve can be stimulated electrically to evoke a potential. This is a reflection of sympathetic cholinergic sudomotor function which induces changes in the resistance of the skin to electrical conduction (Hoeldtke et al, 1992). The main disadvantage is that responses may be difficult to elicit or be habituated and thereby be mistaken as abnormal.

1:6 PATHOGENESIS of AUTONOMIC NEUROPATHY in CYSTIC FIBROSIS

Evidence that autonomic neuropathy exists in cystic fibrosis (CF) comes from several sources.

Davis et al (1980) examined the responses of CF patients to administration of alpha-adrenergic, beta-adrenergic and cholinergic agonists. Firstly, the 11 CF patients had pupillary dilatation of at least 0.5mm in response to significantly lower concentrations of topical phenylephrine compared with healthy controls. Furthermore, there was a significant positive correlation between clinical score and concentration of phenylephrine required for pupillary dilatation. Secondly, 8 CF patients required significantly higher concentrations of isoproterenol to elicit an increase in pulse pressure of at least 22mHg than did controls. This had previously been confirmed in in vitro studies by Davis et al (1978) in which leucocyte preparations from CF patients exhibited markedly lower cAMP responses to isoproterenol than cells from normal controls. Thirdly, Davis et al (1980) demonstrated that the 11 CF patients had pupillary constriction of at least 1mm in the dark in response to much lower concentrations of topical carbachol than did controls. Interestingly the parents of these patients also had similar although less marked responses to these agonists, suggesting that one gene for CF may be sufficient to produce measurable alterations in autonomic sensitivity.

The above results may be explained by the phenomenon of 'denervation hypersensitivity', whereby in autonomic neuropathy, an organ deprived of its innervation becomes more sensitive to the transmitter normally released from those nerves. This 'up-regulation' is thought to be the mediated by an increase in the number and activity of the receptors on the end-organ (Smith, 1992).

More recently, Tattersall et al (2001) investigated cardiovascular autonomic function in 26 adult CF patients using power spectral analysis of heart rate variability by performing a 15 minute modified orthostatic load (this technique has been described earlier as has the concept of reduced heart rate variability in autonomic neuropathy). There were strong correlations between total autonomic function and FEV1 %predicted ($r=0.62$, $p<0.001$) and FVC %predicted ($r=0.68$, $p<0.001$) and these also correlated with both sympathetic and parasympathetic

activity. There was no correlation with the low to high frequency ratio, which the authors suggested would indicate that the autonomic nervous system becomes damaged with increasing disease severity in CF, but that autonomic imbalance is not an important factor in the disease process.

Possible mechanisms for the pathogenesis of autonomic neuropathy in CF

(a) Metabolic causes
(b) Vitamin deficiency
(c) Immunological reasons

(a) Metabolic causes

Lanng et al (1995) conducted a 5 year prospective study of glucose tolerance in CF patients aged above 2 years. The prevalence of diabetes increased from 11% to 24% during the study with an annual age-dependent incidence of 4-9%. Several pathogenic mechanisms may link hyperglycaemia and autonomic neuropathy. One such mechanism is activation of the polyol pathway by glucose via the enzyme aldose reductase (Greene et at, 1992). This results in accumulation of sorbitol and fructose, depletion of myoinositol and slowing of nerve conduction by alteration of neural Na/K ATPase activity or by a disturbance of normal physiological osmoregulatory mechanisms. In fact, a previous pilot study by Green et al (1987) showed that administration of sorbinil (an aldose reductase inhibitor) to 8 diabetics with autonomic neuropathy resulted in improvement of symptoms of neuropathy, decrease in resting mimimum heart rate, improvements in delayed gastric emptying, a decrease in residual urine volume and an improvement in vagally mediated gastric acid secretion.

Another mechanism is nerve ischaemia and hypoxia leading to lipid peroxidation of nerve membranes, a state of oxidative stress; Androne et al (2000) found high levels of lipid peroxide in 10 patients with diabetic autonomic neuropathy.

Treatment with lipoic acid (a powerful inhibitor of iron dependent lipid peroxidation and reaactive oxygen species) significantly reduced lipid peroxide levels.

In addition, Stevens (1995) proposed that metabolic defects in early diabetes could lead to a decrease in the synthesis of nitric oxide in either the vascular endothelium or sympathetic ganglia which would further compromise nerve blood flow.

Autonomic dysfunction also exists in chronic liver disease. Hendrickse and Triger (1992) evaluated autonomic function using standard cardiovascular tests in 104 patients with biopsy-proven liver disease. Cardiovascular autonomic neuropathy was significantly more frequent in advanced liver disease (71.8% Child B or C versus 39.7% Child A, $p<0.001$), but it was independent of aetiology of liver disease. In a separate group of 60 patients with chronic liver disease, the cumulative 4 year mortality rate in patients with vagal neuropathy was 30% compared with 6% in those with normal autonomic function (Hendrickse et al, 1992). Fleckenstein et al (1996) investigated the prevalence of autonomic neuropathy in 33 patients awaiting liver transplantation. 36% had definite and 31% had early neuropathy. Of the former, 14.3%, 31.3% and 60% had Child A, B and C disease respectively. Again, patients with autonomic neuropathy had a significantly higher mortality. More recently, McDougall et al (2002) reported a return to normal peripheral and autonomic function in a 40 year old man with hepatitis-related cirrhosis within 9 months of successive liver transplantation. Winkler and Kempler (2001) have suggested that impairment of axoplasmic transport, thiamine and pyridoxine deficiency, enhanced lipid peroxidation of nerve membranes and circulating immune complexes are important pathogenic factors. It is entirely possible that these have a role to play in cystic fibrosis-related autonomic neuropathy.

(b) **Vitamin deficiency**

Vitamin E is important for the maintenance of normal neurological function in man; in abetalipoproteinaemia and other disorders of fat absorption, early therapy with Vitamin E delays and may prevent the development of neurological complications (Muller et al, 1983). Willison et al (1985) examined 29 CF patients

neurologically but who were asymptomatic. Somatosensory and visual evoked potentials (SSEP and VEP), and electroretinograms (ERG) were performed and the findings correlated with Vitamin E levels. Only 1 patient had definite reflex and sensory abnormalities. Another had abnormal VEP's and ERG's, and a third had abnormal ERG's. All 3 had normal SSEP's. In 2 of the 3 patients with abnormalities, Vitamin E concentrations were below the median value for the whole group.

In 2 CF patients with severe Vitamin E deficiency, the most prominent clinical neurological features were abnormal eye movements, diminished reflexes, decreased vibratory and position sense, ataxia and muscle weakness (Sitrin et al, 1987). Furthermore, treatment with intramuscular injections of Vitamin E partially corrected these deficits. Cynamon et al (1988) performed sural nerve conduction studies in 18 patients wth CF, 8 of whom were deficient in Vitamin E. Sural nerve conduction latency was increased and nerve action potential amplitude decreased in the Vitamin E deficient group. However, the presence of autonomic neuropathy has not previously been investigated in this patient group. Nevertheless, in diabetics, Vitamin E administration improves autonomic function possibly due to its antioxidant effect as shown by Manzella et al (2001). This group examined the effects of Vitamin E on cardiac autonomic function in Type II diabetics as assessed by heart rate variability. There was an increase in the RR interval, total power and high frequency power in these patients.

(c) **Immunological factors**

In 1981, Fraser et al described autoantibodies against the β-receptor in 9 subjects including one with CF. These antibodies were detected by measuring the ability of the plasma to inhibit binding of [^{125}I]iodohydroxybenzylpindolol to calf lung membranes and to precipitate solubilised β-adrenergic receptors of the lung. All 9 subjects had abnormal alpha- and beta-adrenergic and cholinergic responsiveness to phenylephrine, isoproterenol and carbachol respectively. The authors suggested that the beta-adrenergic hyporesponsiveness may have been due to direct blockade of the active site of the receptor by autoantibody.

An immunological association also exists between diabetes and autonomic neuropathy. Gilbey et al (1986) found higher levels of circulating immune complexes in 17 diabetics with autonomic neuropathy compared to controls. Schnell et al (1996) correlated this with autonomic function test results and also utilised a novel technique to assess sympathetic innervation of the heart in a group of Type I diabetics. The patients were assessed for myocardial I^{123}MIBG uptake (this is a noradrenaline anlaogue, the uptake of which is visualised by single photon emission computed tomography and is indicative of sympathetic innervation), ECG-based cardiac autonomic neuropathy and presence of complement-fixing autoantibodies in the cell bodies of cervical sympathetic ganglia. In the group of long-term diabetics the global uptake of I^{123}MIBG negatively correlated with the presence of these autoantibodies. 41% of patients with ECG-based cardiac autonomic neuropathy had autoantibodies. In addition, Vernino et al (2000) demonstrated that high levels of autoantibodies against nicotinic acetylcholine receptors in autonomic ganglia correlated with more severe autonomic dysfunction in patients with a variety of dysautonomias such as paraneoplastic forms, idiopathic gastrointestinal dysmotility and diabetes. Furthermore, levels of these autoantibodies decreased in patients who showed clinical improvement.

1:7 AIMS of the PRESENT RESEARCH

The literature review has shown that autonomic neuropathy is commonly secondary to several chronic diseases such as diabetes, nutritional disorders, malignancy and infections. The aim of this study is to investigate the possibility of autonomic neuropathy in cystic fibrosis. This can be assessed by examining the cardiovascular, ophthalmic, gastrointestinal and urinary systems. It is not possible to examine respiratory autonomic function in CF patients due to structural damage to the end organ, ie the lung, by chronic inflammation.

Cardiovascular system

Autonomic function is traditionally assessed by means of Ewing's tests. However, these have not previously been utilised in CF.

Ewing's tests require patient cooperation and may be insensitive to early changes in sympathetic function. Therefore, there is a need for a simple, sensitive test which requires minimal patient cooperation and which can simultaneously assess parasympathetic and sympathetic function. Spectral analysis of heart rate variability is such a test.

It has been suggested in other chronic diseases that spectral analysis is more sensitive than Ewing's tests in detecting autonomic dysfunction. There is a need to investigate this in CF.

Studies in, for example, diabetes mellitus and chronic congestive cardiac failure have revealed a correlation between the degree of autonomic dysfunction and markers of disease severity. Likewise, it is important to establish this in CF.

Ophthalmic system

The simplest method of assessing pupil function is measurement of pupil diameter percent, an indicator of sympathetic function. This has not previously been examined in CF. Again, there is a need to assess how pupil function may be affected by disease severity in CF.

Gastrointestinal system

Constipation and the distal intestinal obstruction syndrome are frequent problems in CF. However, not all patients experience this. Certainly in diabetics, Battle et al (1980) showed that those patients with severe constipation demonstrated a complete loss of post-prandial myoelectrical activity which could be stimulated pharmacologically. The invasive technique of manometry has traditionally been regarded as the most accurate way of investigating bowel motility. Bowel sounds are generated by peristalsis which is under the control of the autonomic nervous system. Therefore, recording of these utilising a simple, non-invasive technique with no patient discomfort could provide further information regarding bowel motility in CF. Furthermore, how bowel sounds are affected by disease severity could provide insight into why only some patients suffer with constipation or distal intestinal obstruction syndrome.

Urinary system

Incontinence is increasingly being recognised as a significant problem in CF (Orr et al, 2001). There is a need to explore any contribution of autonomic neuropathy towards this.

CHAPTER 2: METHODS

2:1 PATIENTS and CONTROL SUBJECTS

Autonomic nerves innervate virtually every body system; the systems which lend themselves most easily to examination are the cardiovascular, gastro-intestinal, ophthalmic and urinary systems.

Patients

Patients above the age of 16 years attending the Liverpool Adult Cystic Fibrosis Unit were studied. The following markers of disease severity were looked at:

- Age
- FEV1 and FVC (% predicted), oxygen saturations
- Body mass index (BMI)
- HbA1C, fasting glucose concentrations
- Vitamin E levels
- Number of courses of intravenous antibiotics over the preceding 2 years
- Number of days spent on intravenous antibiotics over the preceding 2 years

Controls

These were medical and nursing staff working at the Cardiothoracic Centre, Liverpool. All were over the age of 16 years and without any pre-existing medical conditions.

2:2 CARDIOVASCULAR SYSTEM

Preparation of the subject prior to testing

Subjects abstained from alcohol, caffeine and nicotine for at least 3 hours beforehand as these could potentially affect activity of the autonomic nervous system. They had no evidence of cardiac disease and medications with anticholinergic properties (eg antidepressants, antihistamines), adrenergic antagonists (eg betablockers), sympathomimetic or parasympathomimetic properties were temporarily discontinued 24 hours prior to testing if possible. All patients were in a stable clinical state.

The autonomic function of the cardiovascular system was assessed using two different methods:

1. Traditional Ewing's tests of autonomic function.
2. Power spectral analysis of heart rate variability.

These will now be described in more detail below.

EWING'S TESTS

Subjects were in a relaxed state. The tests were performed at least 1 minute apart to allow the cardiovascular system to return to the resting state. Testing took place at the same time each day in all subjects as there is a diurnal variation to autonomic nervous system function.

The variables measured were **heart rate** and **blood pressure**.

Heart rate reflexes occur within seconds of a disturbance and therefore beat-to-beat analysis is necessary. This was achieved using an ECG machine (Mingograf 7, Siemens-Electra) with a paper speed of 50 mm/sec. The heart rate was calculated as follows:

$$\frac{300}{\text{RR interval as number of large squares between successive R waves}} \times 2$$

If premature beats occurred, both that RR interval and the subsequent one (which may have a compensatory pause) were excluded from the analysis.

Blood pressure was recorded using a standard inflatable sphygmomanometer. The level of measurement was maintained at the level of the heart to avoid hydrostatic pressure effects of the column (Webster et al, 1986).

Intra-arterial catheters may be used to measure beat-to-beat blood pressure change but, although extremely accurate, these are uncomfortable for the patient and not without risk. Modern photoplethysmographic devices (Finapres) generate waveforms similar to intra-arterial recordings but controversy exists as to their accuracy. For example, Kermode et al (1989) demonstrated that Finapres displayed too great a variability for it to be used as an alternative to intra-arterial pressure monitoring in 20 patients during induction of anaesthesia for elective neurosurgical procedures. Idema et al (1989) found that at rest finger systolic blood pressures were higher and finger diastolic and mean arterial pressures were lower than the corresponding intrabrachial pressures in 6 normotensive healthy

male subjects; during exercise, average finger diastolic and mean arterial pressures did not differ from intrabrachial pressures, but systolic pressures increased considerably more than the direct systolic pressure, which potentially limits the use of Finapres during exercise.

Ewing's tests consist of a battery of 5 tests, 3 of which assess parasympathetic and 2 assess sympathetic function.

Tests of parasympathetic function

1. Heart rate variation with respiration (respiratory sinus arrhythmia)
2. Heart rate change in response to the Valsalva manoeuvre
3. Heart rate response to standing upright from the supine position

Tests of sympathetic function

1. Diastolic blood pressure response to sustained handgrip (isometric exercise)
2. Systolic blood pressure response to standing upright from the supine position

Tests of parasympathetic function

1. Heart rate variation with respiration (respiratory sinus arrhythmia)

On inspiration, there is an increase in heart rate and on expiration, a decrease, this being the basis of the respiratory sinus arrhythmia.

The subject was asked to lie supine, with the head elevated at 30°, and breathe deeply at 6 respirations per minute, allowing 5s for inspiration and 5s for expiration (this maximises variation in heart rate [Ravits, 1997]). An electrocardiogram was recorded throughout this period, with a marker to indicate the onset of each inspiration and expiration. It was important that the subject did not hyperventilate as hypocapnia reduces heart rate variation.

Heart rate variation was quantified in 2 ways:

- **Inspiratory – expiratory difference (I-E)**

The maximal (inspiratory) and minimal (expiratory) heart rates with each respiratory cycle were determined from the minimum and maximum RR intervals respectively, and the difference calculated. The value of the mean inspiratory-expiratory difference for the 6 cycles was determined.

- **Expiratory:Inspiratory ratio (E:I)**

The ratio of maximum RR interval during expiration to the minimum RR interval during inspiration was calculated for each respiratory cycle and the average determined.

2. Heart rate response to the Valsalva manoeuvre

The subject was instructed to sit upright and produce a respiratory strain by blowing into a mouthpiece in the form of a 10ml syringe attached to a sphygmomanometer. The strain was maintained for 15s against a pressure of 40mmHg. The system had a slow leak to ensure the patient strained continuously and did not falsely maintain pressure by glottic closure of the airway or tongue occlusion of the mouthpiece (Fig 1). Following cessation of the strain, the patient relaxed and breathed deeply at a normal comfortable rate. The ECG was monitored during the strain and for 30-45s following its release.

The manoeuvre was performed 3 times in total at approximately 1 minute intervals.

The heart rate response was quantified as follows:

- **Valsalva ratio**

The ratio of the maximal RR interval after release of the strain (which is accompanied by a bradycardia) to the minimal RR interval during the period of the strain (which is accompanied by a tachycardia) was calculated. The average of the 3 ratios was then determined to give the Valsalva ratio.

3. Heart rate response to standing upright from the supine position

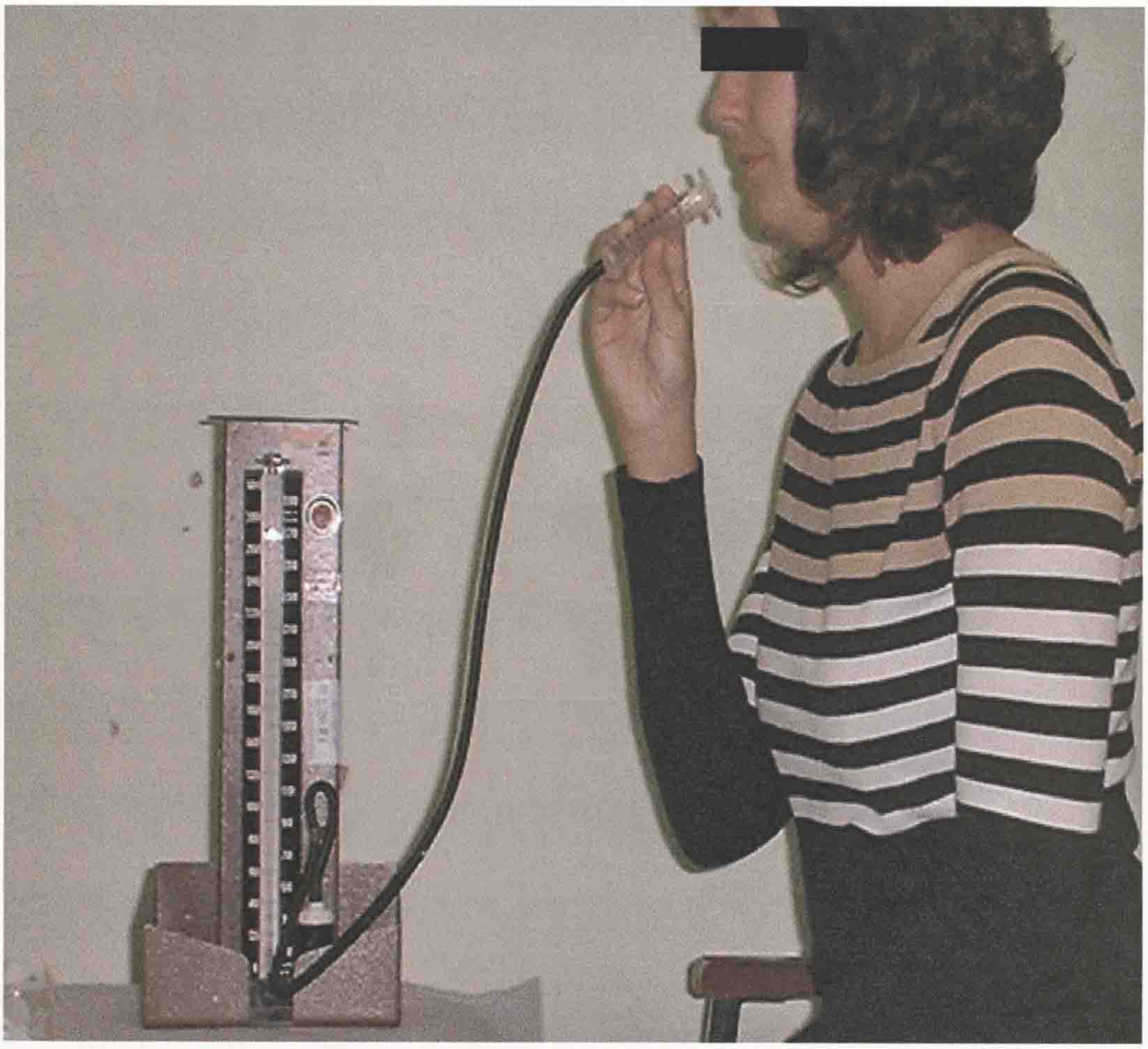
Measurements were obtained after the subject had been in the relaxed supine position for at least 5 minutes. Under continuous ECG monitoring, the patient stood upright as quickly as possible unaided.

The heart rate response was quantified as follows:

- **30:15 ratio**

The ratio of the longest RR interval around the 30^{th} beat after standing to the shortest RR interval around the 15^{th} beat after standing was calculated to give the 30:15 ratio.

Fig 1: Apparatus used to perform the Valsalva manoeuvre



Tests of sympathetic function

1. Diastolic blood pressure response to sustained handgrip (isometric exercise)

With the subject relaxed in the sitting position, the blood pressure was measured in the non-dominant arm at 1 minute intervals on each of 3 occasions and the mean of the 3 diastolic readings taken. The maximum voluntary contraction (MVC) produced by the dominant hand was then determined by asking the subject to quickly squeeze a blood pressure cuff connected to a second sphygmomanometer inflated to 20mmHg. Handgrip was then maintained at 30% MVC for as long as possible for up to 5 minutes. Blood pressure was measured in the non-dominant arm at 1 minute intervals during handgrip.

The blood pressure response was quantified as follows:

- **Diastolic blood pressure difference**

The difference between the highest diastolic blood pressure just before release of the handgrip and the mean of the 3 diastolic readings prior to the test was calculated.

2. Systolic blood pressure response to standing upright from the supine position

Measurements were obtained after the subject had been in the relaxed supine position for at least 5 minutes. The blood pressure was measured on the non-dominant arm 3 times at 1 minute intervals and the mean systolic pressure calculated. The subject then stood up as quickly as possible unaided and the blood pressure was taken immediately.

The blood pressure response was quantified as follows:

- **Systolic blood pressure response**

The postural fall in blood pressure was taken as the difference between the systolic pressures immediately on standing and the mean whilst supine.

Repeatability study

Ewing's tests were carried out on 3 control subjects on 3 successive days at the same time each day to establish repeatability.

POWER SPECTRAL ANALYSIS

Power spectral analysis of the resting heart rate is a relatively new technique which has been used to assess autonomic function in a variety of conditions including diabetes, ischaemic heart disease and chronic liver and renal disease (Freeman et al, 1991).

Investigations in our adult CF patients were performed using the VariaCardio TF4 device (Sima Medical Limited). This consists of an adjustable chest belt with 2 flat electrodes, a built-in UHF transmitter and an on-board MCU-based signal analysis unit. A UHF receiver (with a 30m range) is connected directly to an IBM-PC compatible computer via serial port via an RS-232 connection (Fig 2). A data collection and analysis software package enable the transmitted data to be displayed, stored and edited.

The short-term spectral analysis of the heart rate variability using a modified fast Fourier transform permits a quantification of the influences that individual components of the autonomic nervous system have on the heart rate. A graph known as a power spectrum is generated (Fig 3).

Using the VariaCardio TF4 device, power spectra are calculated by computing the magnitude squared of the modified fast Fourier transform of data points obtained from 300 seconds of tachometer signal.

The subject wearing the adjustable chest belt lay in the supine position for 5 minutes to allow the autonomic nervous system to reach the resting state. ECG recording was commenced for 5 minutes. The subject then assumed the upright for 5 minutes whilst ECG monitoring continued and then became supine again for a final 5 minutes. This demonstrated the reactivity/recovery of the autonomic nervous system back to its baseline.

Throughout the recording period, the subject was completely relaxed, with no distractions and asked to breathe normally; he/she was in a separate room from the investigator and UHF receiver and computer.

Fig 2: Apparatus used for power spectral analysis

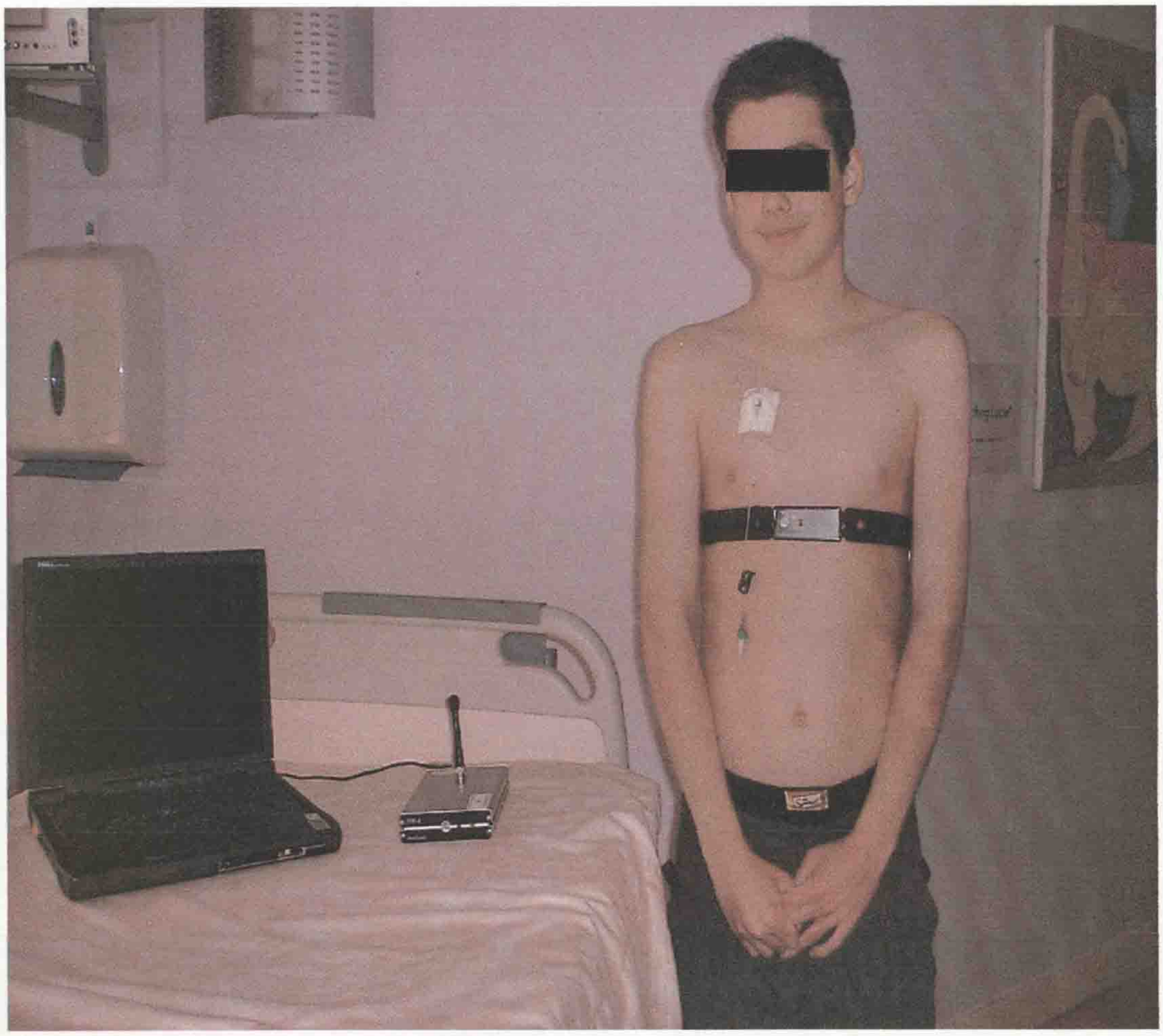
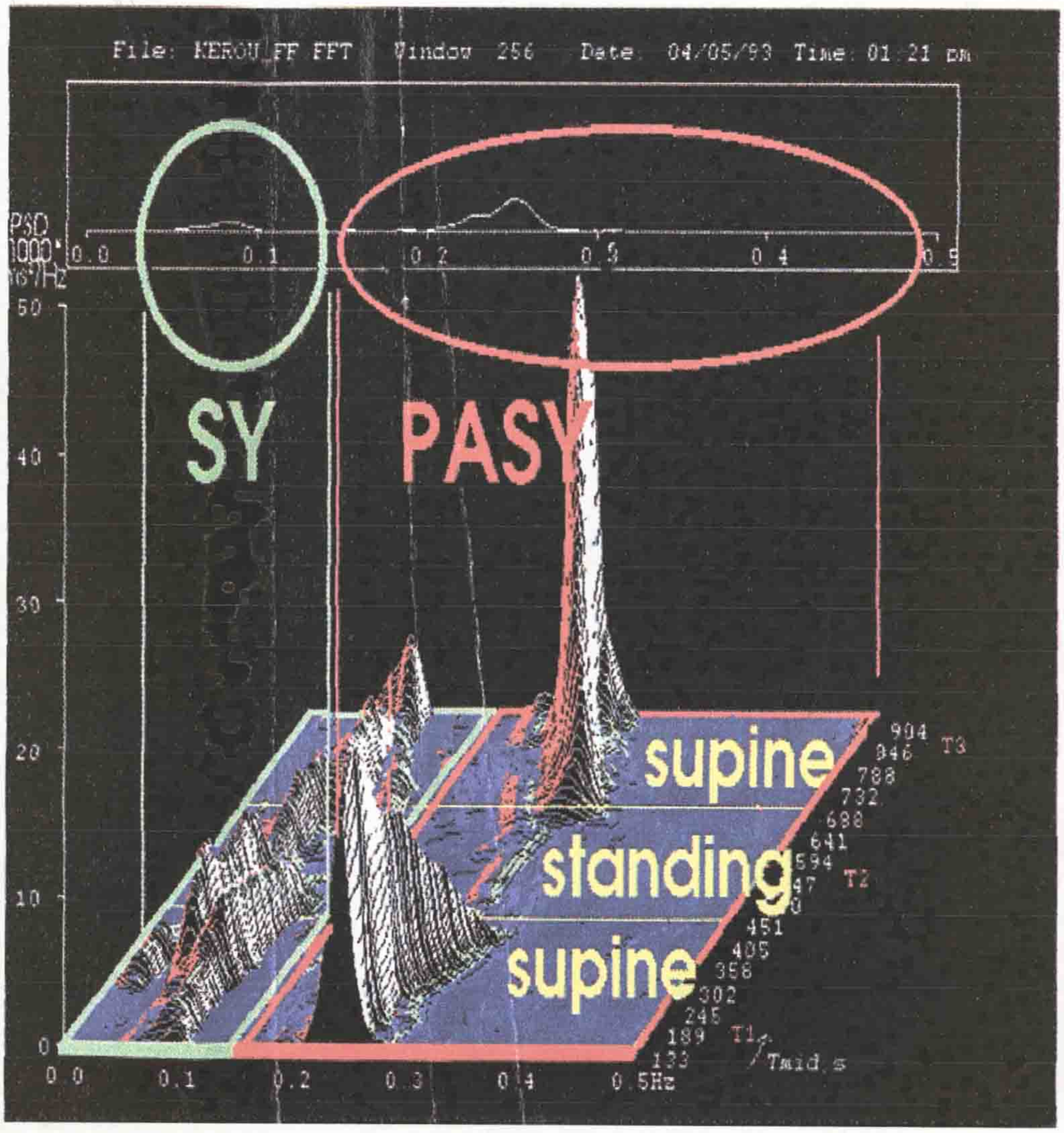


Fig 3: The power spectrum of heart rate variability illustrating sympathetic and parasympathetic components



The results were quantified as follows:

- **Initial resting supine state**
  a) High frequency power-index of parasympathetic activity
  b) Low frequency power-index of sympathetic activity
  c) Low to high frequency ratio-index of sympathovagal balance
  d) Total power-sum of high and low frequency power
  e) RR interval length

- **Assessment over entire 15 minute period**
  Cumulative power-sum of total power in all 3 positions, an index of global autonomic tone.

A significant number of patients were taking inhaled β2-agonist and anticholinergic drugs and therefore, to ensure that the effects of salbutamol and ipratropium had worn off, both of these drugs were administered in doses of 5mg and 0.5mg respectively in nebuliser form through a facemask to a control subject on separate days at the same time each day, and spectral analysis performed at intervals over a 3 hour period.

As there is no established range of normal values, a control group was also included in the study.

Repeatability study

Power spectral analysis was carried out on 3 subjects on 3 successive days at the same time each day.

2:3 OPHTHALMIC SYSTEM

The method of pupil darkness diameter percent as a measure of sympathetic function was used.

Subject preparation was as described for the cardiovascular system.

The subject placed his/her head on a support with a millimetre scale held under the left eye as a reference (Fig 4). The room was then completely blacked out. After 30s (this allows sufficient time for parasympathetic tone to disappear and sympathetic tone to plateau [Smith and Dewhirst, 1986]), a flash photograph of the left eye was taken with the subject staring into infinity, with a digital camera (Fujifilm MX-1200, 1.3 megapixels, resolution 1,280 X 960 pixels) at a distance of 30cm. The photograph was then downloaded onto a Fujifilm PictureShuttle application on a Microsoft Windows NT Workstation. The images could thus be magnified and their contrast/brightness adjusted to facilitate measurement of pupil darkness diameter percent.

Control subjects were used as a comparison with patients.

To ensure that there were no topical effects on the pupil from nebulised salbutamol or ipratropium, these drugs were admninistered via a nebuliser and facemask to a control subject and the left eye photographed beforehand, immediately after drug administration, and at 15 minute intervals for up to 30 minutes.

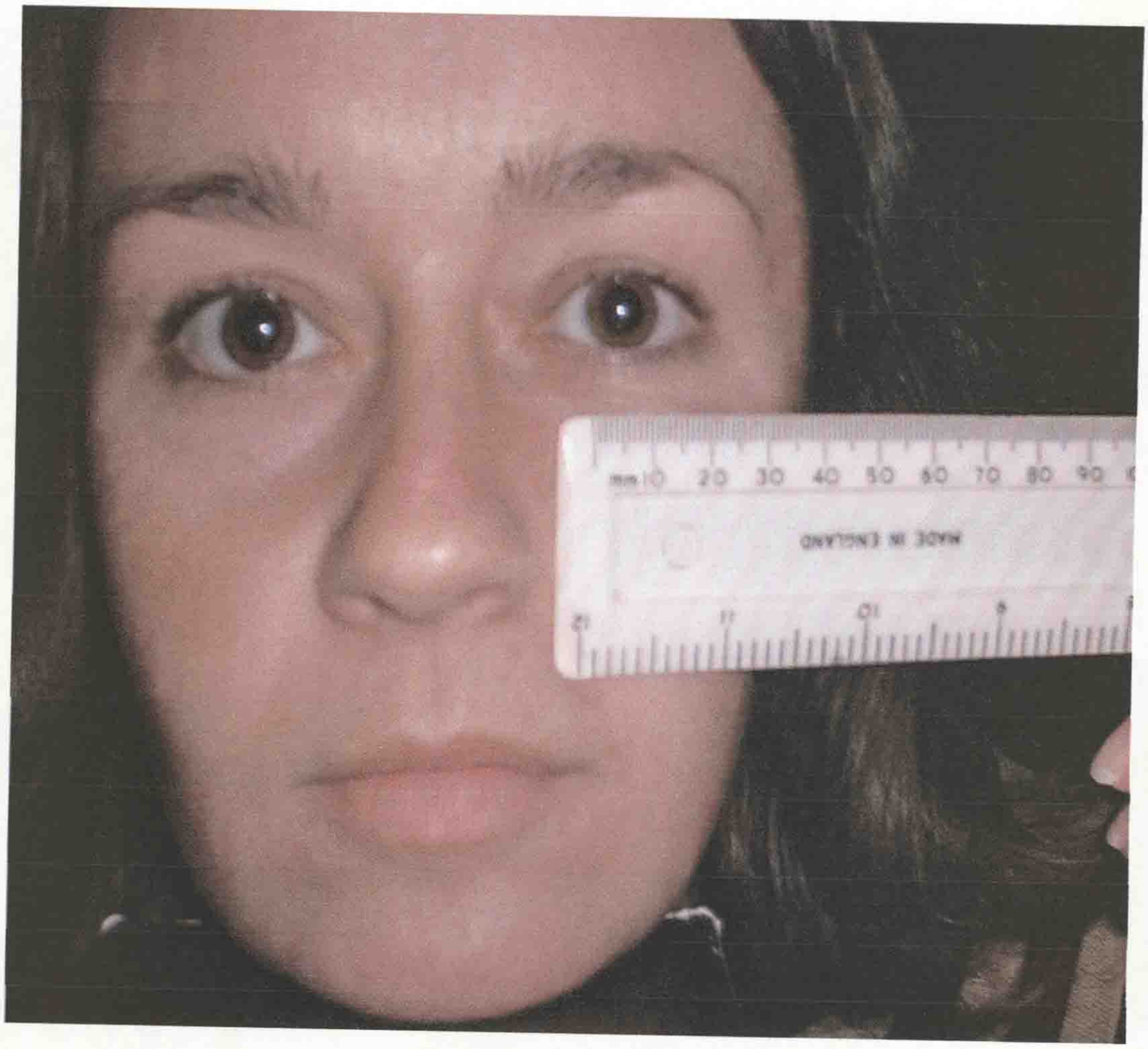
Reproducibility study

This was performed on a group of control subjects. The left eye was photographed twice 15 minutes apart.

Repeatability study

The left eye of a group of control subjects was photographed twice one week apart.

Fig 4: Positioning of the patient for photographing the eye



2:4 GASTROINTESTINAL SYSTEM

Recording and analysis of bowel sounds

Subject preparation (for both patients and controls) was as described for the cardiovascular system. In addition, no patients on opiates were included.
The subject was fasted for at least 3 hours prior to testing in the supine position. Bowel sounds were recorded for remote analysis using a portable digital audio tape recorder (Sony Digital Audio Tape Recorder TCD-D) and hybrid stethoscope-microphone arrangement taped with surgical Micropore tape to the left lower abdomen (Fig 5). All recordings were taken in a quiet area to minimise pollution from unwanted extraneous sounds.

The recordings were analysed in 2 ways:

- Using the Physiological Signal Analysis System (PhiSAS)
- Using a wave editor programme called 'CoolEdit'

Physiological Signal Analysis System

The recordings were transferred from DAT tape to a sound analysis system known as PhiSAS (Physiological Signal Analysis System) via hardware signal conditioning (low-pass filtering at 22 kHz, and analogue-to-digital sampling at 44 kHz). The signals were then further post-processed (in software) ie down-sampled to respective sets of spectral bandwidths of 4, 2 and 1 kHz.

PhiSAS is a novel system that was originally developed for the observation of lung sounds. The system consists of a personal computer supported with dedicated software and hardware. PhiSAS utilises spectral analysis to analyse the spectral construction of sound, providing information on the median frequency and power (energy) of the bowel sounds.

CoolEdit

CoolEdit (Syntrillium Software Corporation 2000) is a programme with an audio function used widely by the music industry.

The bowel sounds were converted into wave format (using PhiSAS as above) and analysed using CoolEdit. The number of sounds with amplitude 30% above baseline (this best discriminated between patients and controls) were counted for the whole recording, thus allowing the number of sounds per minute to be calculated. Fig 6 shows that a bowel sound typically exhibits an exponential decay pattern.

Comparisons were made between normal subjects, non-constipated CF patients and constipated CF patients. In addition, this latter group was also been analysed in the non-constipated state.

Repeatability study

Bowel sounds were recorded from 2 control subjects on 3 successive days.

Fig 5: Apparatus used to record bowel sounds

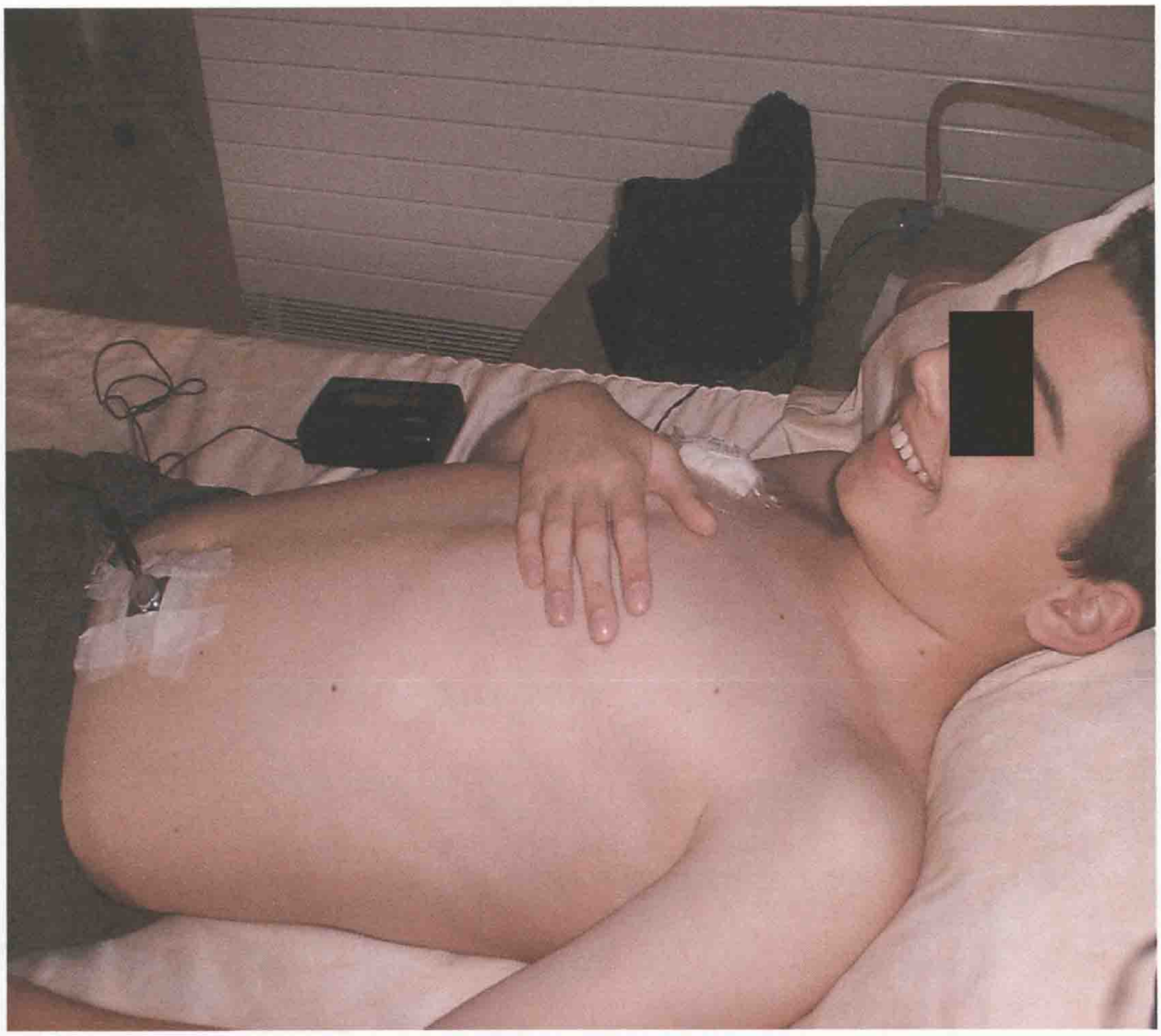
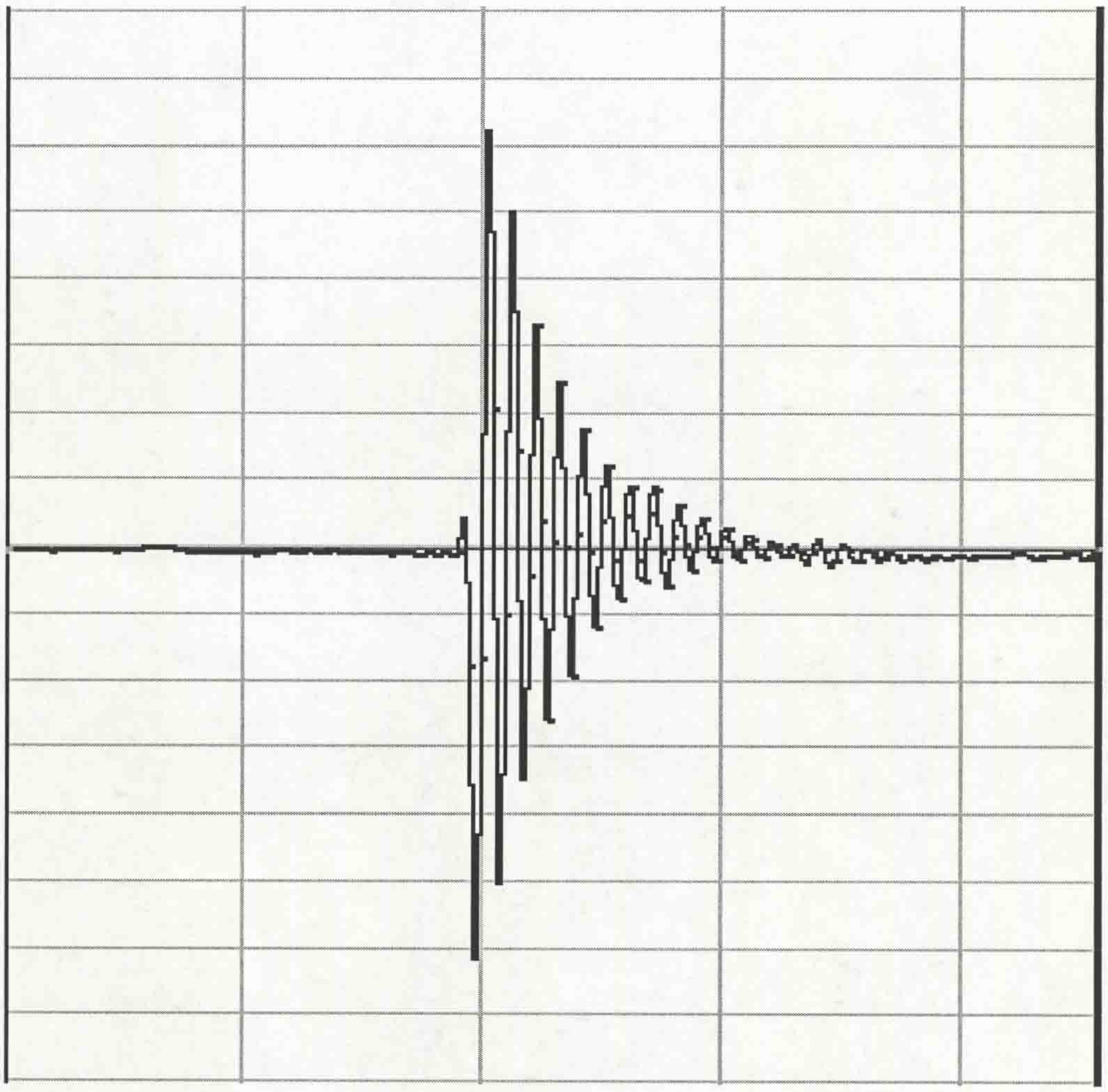


Fig 6: Typical exponential decay pattern of a bowel sound



2:5 URINARY SYSTEM

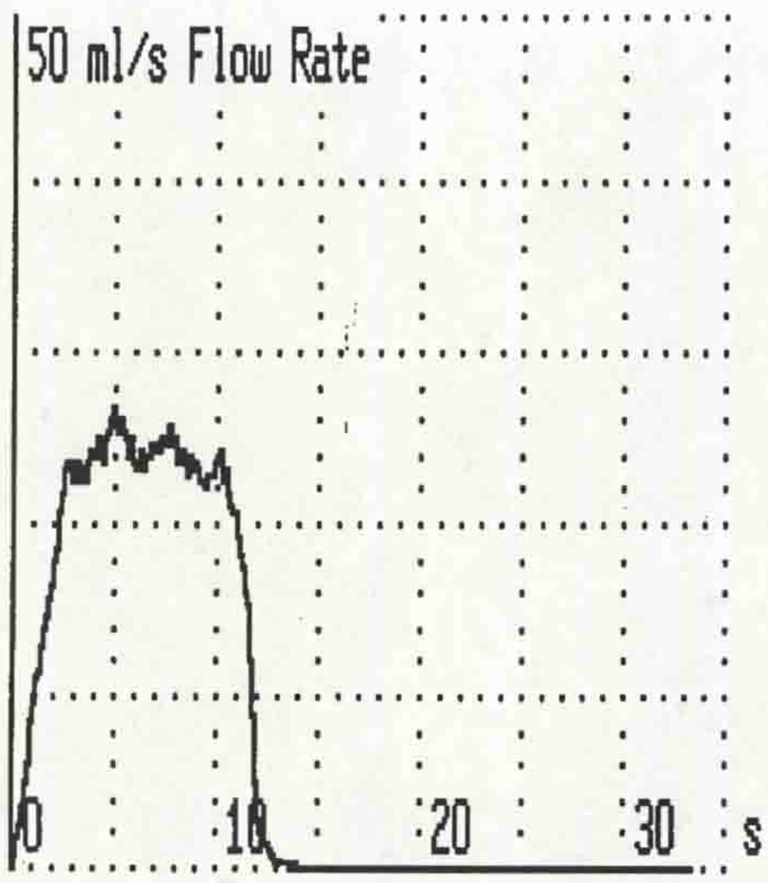
Simple uroflowmetry studies were performed on female patients using a portable flowmeter.

On the morning of the test, the subject was asked to drink more fluid than usual to ensure a full bladder. The patient then micturated into the flowmeter which calculated values for maximum flow rate, average flow rate and voided volume. A graphic trace of urine flow was also printed (example Fig 7). Ultrasound examination of the bladder was then performed to evaluate the post-micturition residual volume of urine.

The study of voiding in men and women has previously been handicapped by the lack of a reference range defining normal limits. In 1989, Haylen et al established nomogram charts for both sexes in centile form, for both the maximum and average urine flow rates over a wide range of voided volumes (15-600 mls) using statistical transformation of the data. In addition, these investigators reported that in women flow rate did not depend on age or parity. Figs 8 and 9 are the so-called Liverpool nomograms for maximum and average urine flow rates in women (the age range studied was 16 to 63 years).

Due to the discomfort associated with more complex filling and voiding cystometry studies, all patients declined these.

Fig 7: Example of urine flow trace



Results of UROFLOWMETRY

Voiding Time	T100	13	s
Flow Time	TQ	13	s
Time to max Flow	TQmax	5	s
Max Flow Rate	Qmax	26.7	ml/s
Average Flow Rate	Qave	18.7	ml/s
Voided Volume	Vcomp	246	ml

Fig 8: Liverpool nomogram showing maximum urine flow rates in women

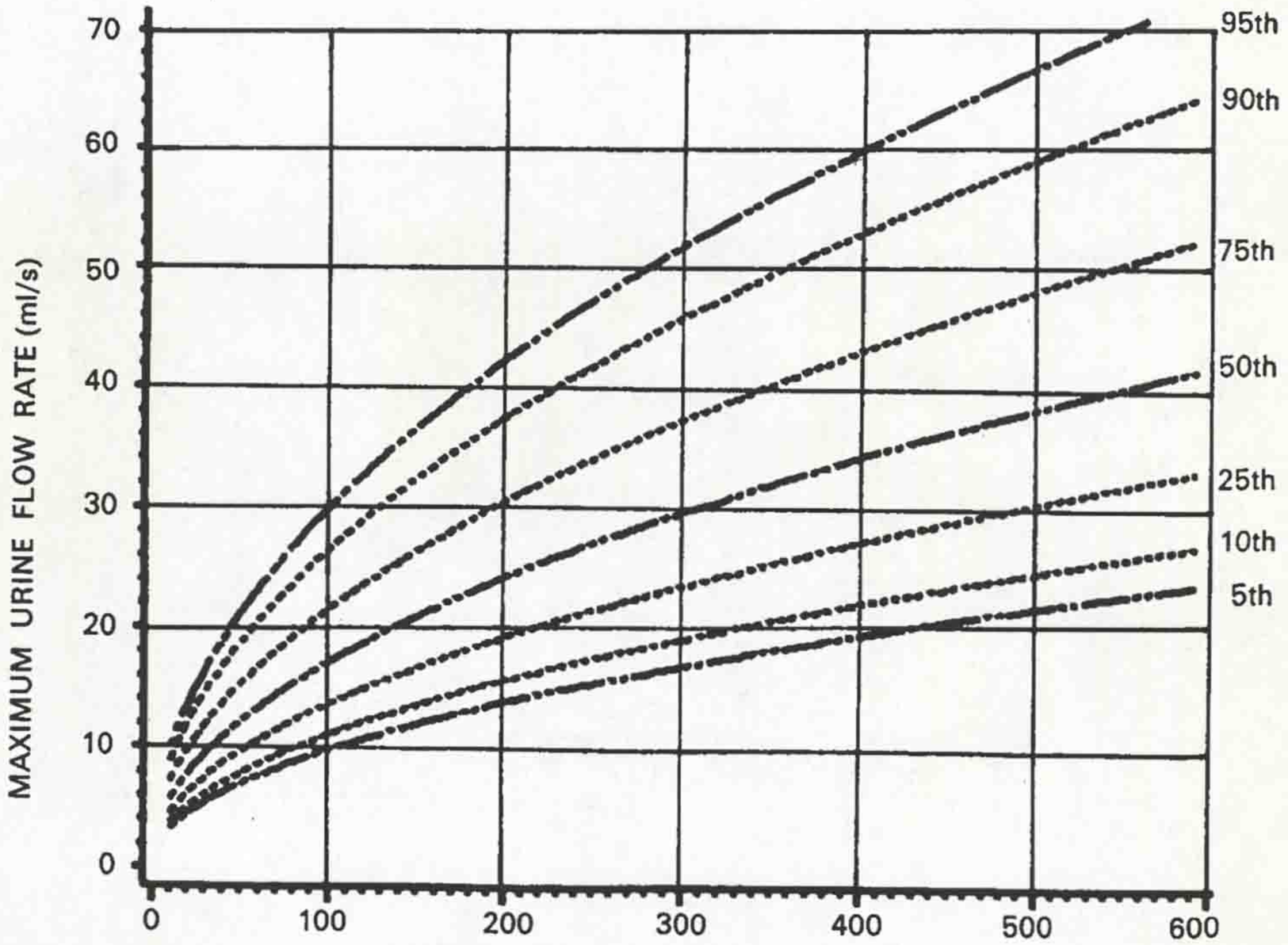
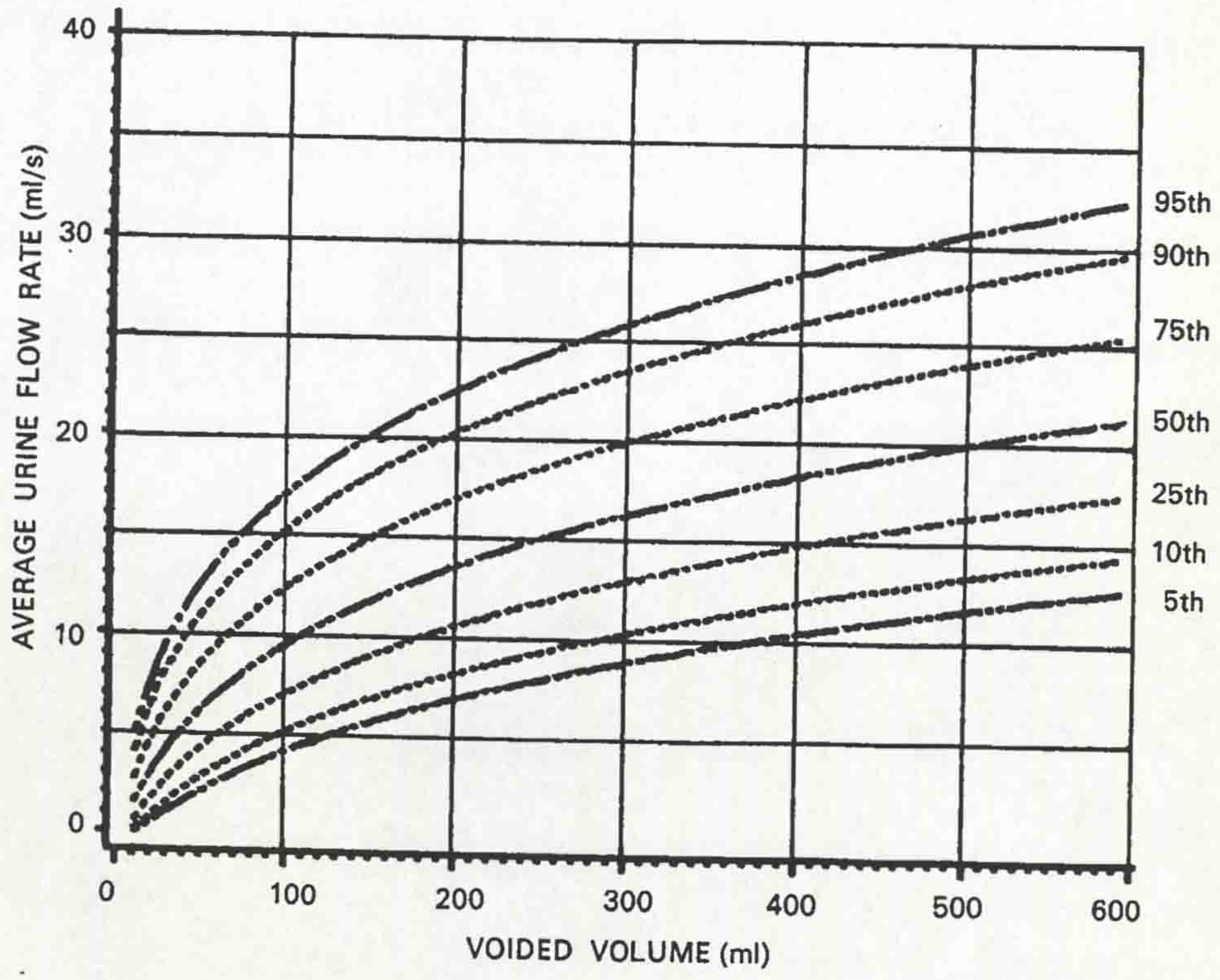


Fig 9: Liverpool nomogram showing average urine flow rates in women



2:6 STATISTICAL METHODS

Differences between the mean values of the various patient groups were tested using the Student's t test as the data showed a near normal distribution.

Correlation coefficients were used to correlate the results of the variables with markers of disease severity.

A value of probability less than 0.05 was regarded as significant.

Intra-individual coefficients of variation, as a measure of reproducibility, were calculated as follows:

$$\frac{\text{standard deviation of observations}}{\text{mean of observations}} \times 100$$

2:7 ETHICAL APPROVAL

Full ethical approval for this study was obtained from both the Liverpool Research Ethics Committee and the Academic Committee at the Cardiothoracic Centre, Liverpool. Informed, written consent was also obtained from all patients involved.

CHAPTER 3: CARDIOVASCULAR SYSTEM (I)

3:1 EWING'S TESTS: RESULTS

3:2 EWING'S TESTS: DISCUSSION

3:1 EWING'S TESTS: RESULTS

Control subjects

10 control subjects participated in the study; 2 male and 8 female. The mean age was 30 years (range 22-34 years).

Table 1 shows the mean value and range for each of the Ewing's tests; in all cases the mean values are in the normal range as defined by Ewing and Clarke (1982). For some control subjects, the values for some of the Ewing's tests were borderline or abnormal.

In order to establish the repeatability of each of the Ewing's tests, measurements were made on 3 control subjects on 3 successive days. The mean coefficient of variation was calculated for each test as follows:

$$\text{coefficient of variation} = \frac{\text{standard deviation}}{\text{mean}} \times 100$$

The results are shown in Tables 2(a) – 2(g). The mean coefficients of variation for the inspiratory-expiratory difference (I-E), E:I ratio, Valsalva ratio, 30:15 ratio, diastolic blood pressure change on isometric exercise and systolic blood pressure change on standing upright were 17.1%, 5.44%, 8.1%, 6.75%, 69.62% and 10.47% respectively.

Table 3 shows the normal, borderline and abnormal values for each of the Ewing's tests (Ewing and Clarke, 1982).

For each of the tests, the proportion of subjects falling into each of normal, borderline and abnormal categories was determined. As expected the majority of subjects had normal results (Table 4).

The results of tests of autonomic function are said to decline with age. I found that only the inspiratory-expiratory difference and systolic blood pressure change during orthostatic load correlated negatively with age ($P<0.05$ for both) (Figs 1 and 2).

Table 1: Mean values for Ewing's tests in the control group

EWING'S TEST	NORMAL RANGE (FROM LITERATURE)	MEAN VALUE FOR CONTROL GROUP (RANGE)
I-E (beats per minute)	>15	21.3 (12.9-30.9)
E:I	>1.2	1.35 (1.18-1.56)
Valsalva ratio	>1.21	1.47 (1.02-1.97)
30:15	>1.04	1.48 (1.22-1.90)
Diastolic blood pressure rise during isometric exercise (mmHg)	>16	23.49 (6.7-40.0)
Systolic blood pressure fall during orthostatic load (mmHg)	<10	14.5 (6.7-26.7)

Abbreviations

I-E Inspiratory-expiratory difference

E:I Ratio of maximum RR interval during expiration to minimum RR interval during inspiration

30:15 Ratio of 30^{th} to 15^{th} RR intervals after standing

Table 2(a): Coefficients of variation (CV) for I-E (beats/minute) in 3 control subjects

Subject	I-E day 1	I-E day 2	I-E day 3	Mean	Standard deviation	CV (%)
1	20.55	23.47	17.6	20.54	2.935	14.289
2	24.5	28.1	23.1	25.233	2.579	10.221
3	14.0	24.4	20.1	19.5	5.226	26.8

Mean CV = 17.1%

Table 2(b): Coefficients of variation (CV) for E:I in 3 control subjects

Subject	E:I day 1	E:I day 2	E:I day 3	Mean	Standard deviation	CV (%)
1	1.35	1.36	1.26	1.323	0.055	4.157
2	1.41	1.5	1.34	1.417	0.08	5.646
3	1.22	1.39	1.3	1.303	0.085	6.523

Mean CV = 5.44%

Table 2(c): Coefficients of variation (CV) for the Valsalva ratio (VR) in 3 control subjects

Subject	VR day 1	VR day 2	VR day 3	Mean	Standard deviation	CV (%)
1	1.82	1.99	1.53	1.78	0.233	13.09
2	1.97	1.83	2.05	1.95	0.111	5.692
3	1.27	1.15	1.16	1.193	0.066	5.532

Mean CV = 8.10%

Table 2(d): Coefficients of variation (CV) for the 30:15 ratio in 3 control subjects

Subject	30:15 day 1	30:15 day 2	30:15 day 3	Mean	Standard deviation	CV (%)
1	1.1	1.22	1.21	1.177	0.067	5.692
2	1.34	1.23	1.46	1.343	0.115	8.563
3	1.26	1.17	1.32	1.25	0.075	6.0

Mean CV = 6.75%

Table 2(e): Coefficients of variation (CV) for diastolic blood pressure change (mmHg) (DBP) on isometric exercise in 3 control subjects

Subject	DBP $_{day\ 1}$	DBP $_{day\ 2}$	DBP $_{day\ 3}$	Mean	Standard deviation	CV (%)
1	13.3	0	18.3	10.533	9.458	89.794
2	6.7	13.3	1.7	7.233	5.818	80.437
3	18.3	40	26.7	28.333	10.942	38.619

Mean CV = 69.62%

Table 2(f): Coefficients of variation (CV) for systolic blood pressure change (mmHg) (SBP) on standing in 3 control subjects

Subject	SBP $_{day\ 1}$	SBP $_{day\ 2}$	SBP $_{day\ 3}$	Mean	Standard deviation	CV (%)
1	10	10	8.3	9.433	0.981	10.4
2	25	23.3	25	24.433	0.981	4.015
3	8.3	10	11.7	10	1.7	17

Mean CV = 10.47%

Table 2(g): Summary of mean coefficients of variation (CV) for Ewing's tests in 3 control subjects

EWING'S TEST	COEFFICIENT OF VARIATION (%)
I-E	17.1
E:I	5.44
Valsalva ratio	8.10
30:15	6.75
Diastolic blood pressure rise during isometric exercise	69.62
Systolic blood pressure fall during orthostatic load	10.47

Abbreviations

I-E Inspiratory-expiratory difference

E:I Ratio of maximum RR interval during expiration to minimum RR interval during inspiration

30:15 Ratio of 30^{th} to 15^{th} RR intervals after standing

Table 3: Normal, borderline and abnormal values for Ewing's tests

EWING'S TEST	NORMAL RANGE	BORDERLINE RANGE	ABNORMAL RANGE
Heart rate variation on deep breathing: I-E (bpm) E:I	 >15 >1.2	 11-14 -	 <10 <1.2
Heart rate response to standing (30:15)	>1.04	1.11-1.03	<1.00
Heart rate response to the Valsalva manoeuvre (Valsalva ratio)	>1.21	1.11-1.20	<1.10
DBP rise during isometric exercise (mmHg)	>16	11-15	<10
SBP fall on standing upright (mmHg)	<10	11-29	>30

Abbreviations

DBP Diastolic blood pressure

SBP Systolic blood pressure

Table 4: Proportion of control subjects (n = 10) with normal, borderline and abnormal results for Ewing's tests

EWING'S TEST	NUMBER NORMAL (%)	NUMBER BORDERLINE (%)	NUMBER ABNORMAL (%)
I-E	8 (80)	2 (20)	0 (0)
E:I	9 (90)	0 (0)	1 (10)
30:15	10 (100)	0 (0)	0 (0)
Valsalva ratio (n = 9)	6 (67)	2 (22)	1 (11)
Diastolic blood pressure rise during isometric exercise (mmHg)	7 (70)	1 (10)	2 (20)
Systolic blood pressure fall during orthostatic load (mmHg)	10 (100)	0 (0)	0 (0)

Abbreviations

I-E Inspiratory-expiratory difference

E:I Ratio of maximum RR interval during expiration to minimum RR interval during inspiration

30:15 Ratio of 30^{th} to 15^{th} RR intervals after standing

Fig 1: Correlation between inspiratory-expiratory (I-E) difference and age in 10 control subjects.

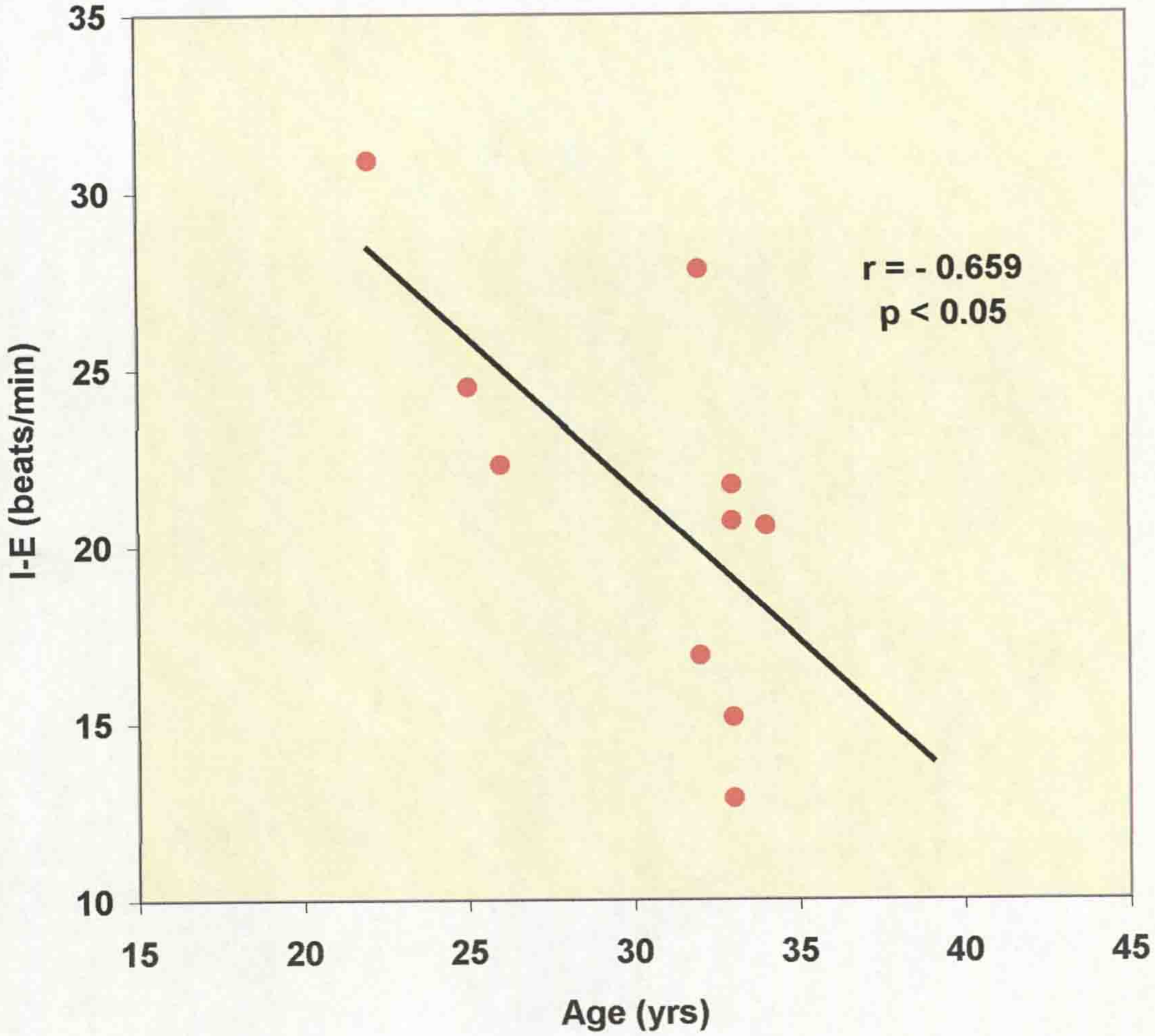
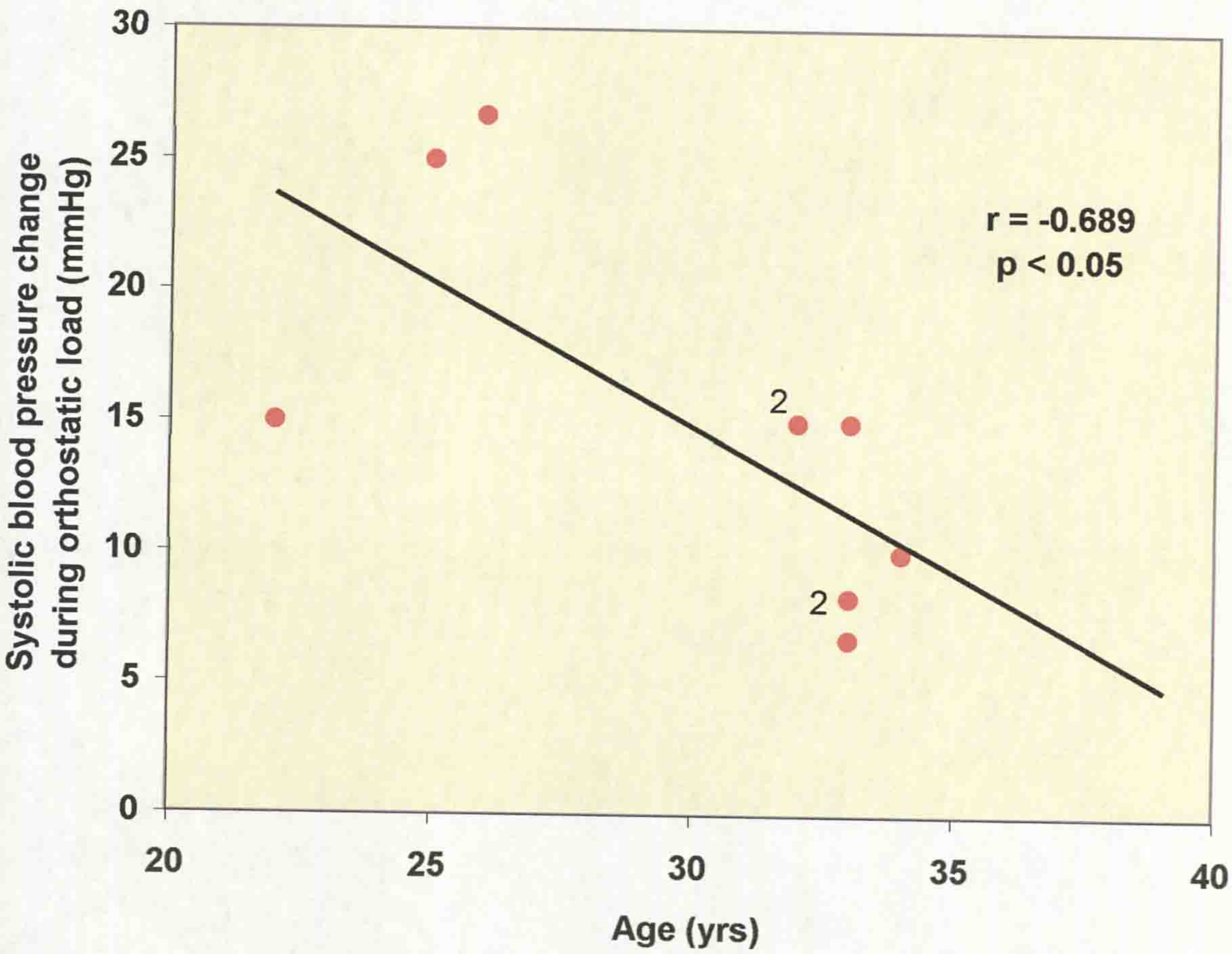


Fig 2: Correlation between systolic blood pressure change during orthostatic load in 10 control subjects.



Patients

38 patients were included, 18 male and 20 female. Their mean age was 24 years (range 17-41 years). All patients were in a stable clinical state and tested at the same time each day. Only 7 out of 38 patients (18.4%) were able to perform the Valsalva manoeuvre. This was not readily explained by worse pulmonary function or differences in any other marker of disease severity (see below) but may have been a consequence of many of the patients coughing during the expiratory phase of this forced manoeuvre, resulting in an inability to maintain a pressure of 40mmHg for 15 seconds.

The following markers of disease severity were determined: spirometric indices, body mass index, fasting glucose, HbA1c, Vitamin E levels, number of courses of intravenous antibiotics and number of days spent on intravenous antibiotics over the preceding 2 years. Mean values and ranges are shown in Table 5. All mean values for Ewing's tests except the diastolic blood pressure change during isometric exercise were in the normal range (Table 6).

The percentage of patients with results below the lower end of normal varied from 0% (Valsalva ratio) to 50% (diastolic blood pressure change during isometric exercise) (Table 7). However, it should be remembered that the latter demonstrates the poorest reproducibility.

Only the expiratory:inspiratory ratio correlated significantly with age ($p<0.01$) as shown by Fig 3.

It was postulated that the group of patients unable to perform the Valsalva manoeuvre (n=31) would have significantly worse mean values for the remaining Ewing's tests as well as markers of disease severity than those patients unable to perform the manoeuvre (n=7). However, this was found not to be the case; Tables 8 and 9 compare the mean values for the 2 groups. In all cases p=NS.

Table 5: Mean values for markers of disease severity in the patient group

PARAMETER	NORMAL RANGE (WHERE APPLICABLE)	MEAN VALUE (RANGE)
FEV1 (% predicted)	-	55.4 (17-125)
FVC (% predicted)	-	71.0 (29-128)
O_2 saturation (%)	-	96.4 (87-100)
Body mass index (BMI) (kg/m^2)	-	20.0 (13.9-31.2)
Fasting glucose (mmol/l)	3.5-5.5	6.7 (3.4-24.8)
HbA1c (%)	<7.0	6.4 (5.1-10.7)
Vitamin E (umol/l)	14-47	13.1 (1-38)
No of courses of iv antibiotics over previous 2 yrs	-	7.2 (1-21)
No of days on iv antibiotics over previous 2 yrs	-	113.2 (7-351)

Table 6: Mean values for Ewing's tests in the patient group

EWING'S TEST	MEAN VALUE (RANGE)
I-E (beats per minute)	21.5 (8.2-35.1)
E:I	1.32 (1.12-1.74)
Valsalva ratio	1.70 (1.07-2.39)
30:15	1.24 (1.0-1.88)
Diastolic blood pressure change during isometric exercise (mmHg)	12.4 (-6.7-30)
Systolic blood pressure change during orthostatic load (mmHg)	0.2 (-17.5-18.3)

Abbreviations

I-E Inspiratory-expiratory difference

E:I Ratio of maximum RR interval during expiration to minimum RR interval during inspiration

30:15 Ratio of 30^{th} to 15^{th} RR intervals after standing

Table 7: Proportion of patients with abnormal values for Ewing's tests (below lower end of normal)

EWING'S TEST	NUMBER BELOW LOWER END OF NORMAL (%)
I-E	4/38 (10.5)
E:I	8/38 (21.1)
Valsalva ratio	0/7 (0)
30:15	4/38 (10.5)
Diastolic blood pressure change during isometric exercise	19/38 (50)
Systolic blood pressure change during orthostatic load	0/38 (0)

Abbreviations

I-E Inspiratory-expiratory difference

E:I Ratio of maximum RR interval during expiration to minimum RR interval during inspiration

30:15 Ratio of 30^{th} to 15^{th} RR intervals after standing

Fig 3: Correlation between expiratory:inspiratory (E:I) ratio and age in 38 patients

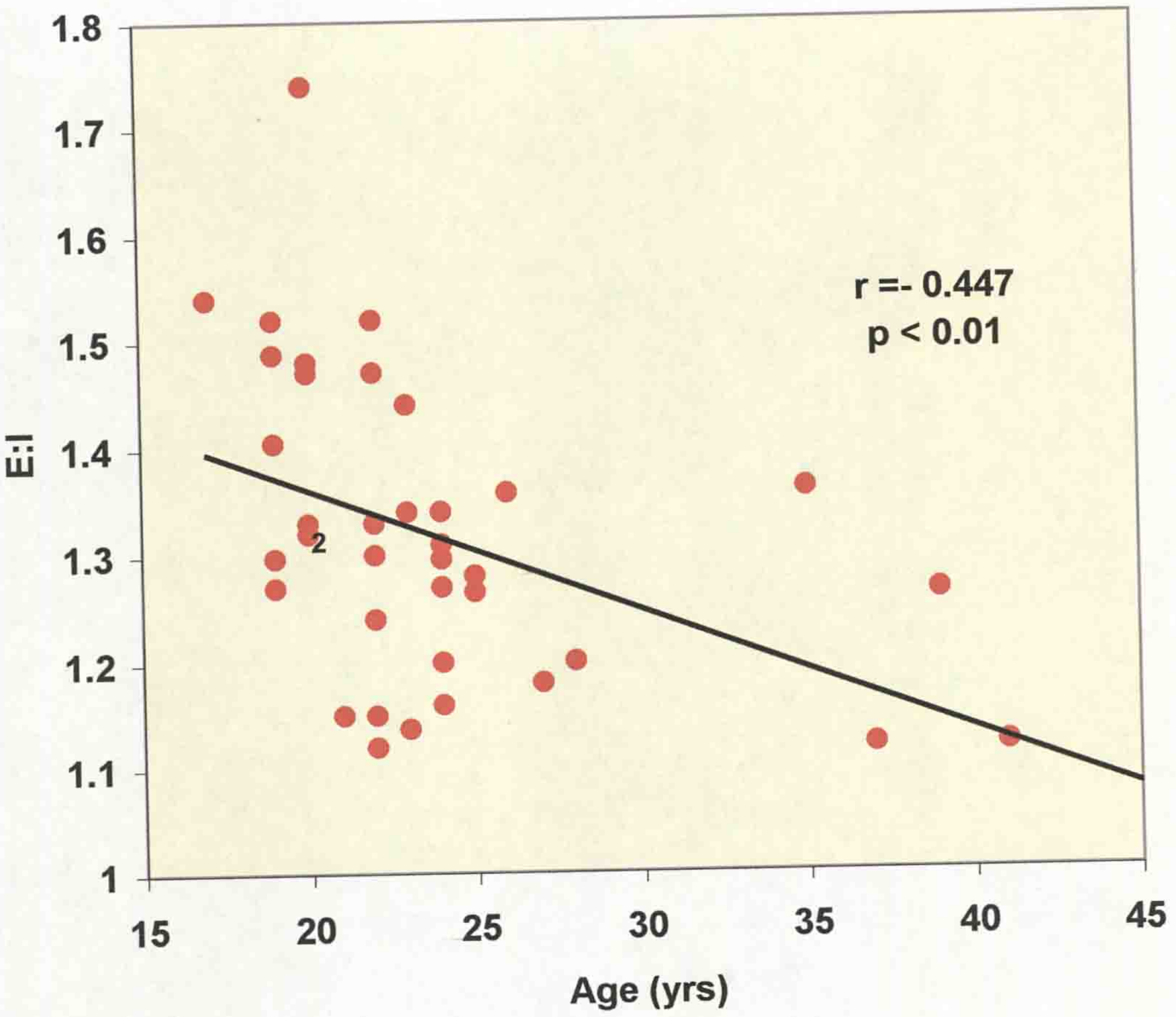


Table 8: Comparison between the Valsalva (n=7) and non-Valsalva (n=31) groups for Ewing's tests

EWING'S TEST	MEAN VALUE FOR VALSALVA GROUP (RANGE)	MEAN VALUE FOR NON-VALSALVA GROUP (RANGE)	P VALUE
I-E (beats per minute)	21.0 (8.2-35.1)	21.6 (8.6-32.9)	0.857
E:I	1.34 (1.15-1.74)	1.31 (1.12-1.54)	0.758
30:15	1.22 (1.04-1.5)	1.24 (1-1.88)	0.784
Diastolic blood pressure change during isometric exercise (mmHg)	11.4 (6.7-21.7)	12.6 (-6.7-30)	0.670
Systolic blood pressure change during orthostatic load (mmHg)	0.7 (-15-18.3)	-0.4 (-17.5-15)	0.810

Abbreviations

I-E Inspiratory-expiratory difference

E:I Ratio of maximum RR interval during expiration to minimum RR interval during inspiration

30:15 Ratio of 30^{th} to 15^{th} RR intervals after standing

Table 9: Mean values for markers of disease severity for the Valsalva and non-Valsalva groups

PARAMETER	MEAN VALUE FOR VALSALVA GROUP (RANGE)	MEAN VALUE FOR NON-VALSALVA GROUP (RANGE)	P VALUE
Age (yrs)	22.7 (20-25)	21.0 (17-41)	0.363
FEV1 (% predicted)	59.4 (24-125)	54.4 (17-88)	0.723
FVC (% predicted)	77 (45-128)	69.6 (29-106)	0.495
O_2 saturations (%)	97 (95-99)	96.2 (87-100)	0.266
BMI (kg/m^2)	19.0 (13.9-23.7)	20.3 (14.7-31.2)	0.449
Fasting glucose (mmol/l)	6.0 (4.4-11.9)	6.8 (3.4-24.8)	0.564
HbA1c (%)	6.1 (5.7-7)	6.4 (5.1-10.7)	0.195
Vitamin E (umol/l)	10.6 (4-17)	14.0 (1-38)	0.272
No of courses of iv antibiotics over previous 2 yrs	8.7 (2-17)	6.9 (1-21)	0/450
No of days on iv antibiotics over previous 2 yrs	142.0 (22-351)	104.4 (7-238)	0.439

Comparison between control and patient groups

When comparing Ewing's tests' results between control subjects and patients, the 30:15 ratio, diastolic blood pressure change during isometric exercise and systolic blood pressure change on standing indicated significant differences ($p<0.004$, $p<0.02$) and $p<0.001$respectively) (Table 10).

Comparison between patients colonised with *Pseudomonas aeruginosa* and *Burkholderia cepacia*

27 patients were colonised by *Pseudomonas aeruginosa* only and 11 were colonised by *Burkholderia cepacia* only. There were no significant differences for Ewing's tests results between these groups (Table 11). No *cepacia* patients were able to perform the Valsalva manoeuvre and so this comparison cannot be made.

Comparison between male and female patients

Only the diastolic blood pressure change during isometric exercise revealed a significant difference ($p<0.013$) between male ($n=18$) and female ($n=20$) patients. Tables 12 and 13 show Ewing's tests' results and markers of disease severity for these 2 groups.

Table 10: Comparison between control subjects and CF patients for Ewing's tests

EWING'S TEST	MEAN VALUE FOR CONTROLS (RANGE)	MEAN VALUE FOR PATIENTS (RANGE)	P VALUE
I-E (beats per minute)	21.3 (12.9-30.9)	21.5 (8.2-35.1)	0.936
E:I	1.35 (1.18-1.56)	1.32 (1.12-1.74)	0.445
Valsalva ratio	1.47 (1.02-1.97)	1.70 (1.07-2.39)	0.251
30:15	1.48 (1.22-1.90)	1.24 (1.0-1.88)	0.004
Diastolic blood pressure change during isometric exercise (mmHg)	23.5 (6.7-40)	12.4 (-6.7-30.0)	0.020
Systolic blood pressure change during orthostatic load (mmHg)	14.5 (6.7-26.7)	-0.2 (-17.5-18.3)	< 0.001

Abbreviations

I-E Inspiratory-expiratory difference

E:I Ratio of maximum RR interval during expiration to minimum RR interval during inspiration

30:15 Ratio of 30^{th} to 15^{th} RR intervals after standing

Table 11: Comparison of Ewing's tests results between patients colonised with *Pseudomonas aeruginosa* (n=27) and *Burkholderia cepacia* (n=11)

EWING'S TEST	MEAN VALUE FOR *Psa* PATIENTS (RANGE)	MEAN VALUE FOR *B cepacia* PATIENTS (RANGE)	P VALUE
I-E (beats/minute)	20.9 (8.2-35.1)	22.2 (15.4-32.3)	0.569
E:I	1.32 (1.12-1.74)	1.29 (1.15-1.44)	0.409
30:15	1.24 (1.0-1.61)	1.18 (1.0-1.73)	0.421
Diastolic blood pressure change during isometric exercise (mmHg)	12.63 (0-30)	11.2 (-6.7-30)	0.907
Systolic blood pressure change during orthostatic load (mmHg)	-0.03 (-17.5-18.3)	0.24 (-10-5)	0.683

Abbreviations

I-E Inspiratory-expiratory difference

E:I Ratio of maximum RR interval during expiration to minimum RR interval during inspiration

30:15 Ratio of 30^{th} to 15^{th} RR intervals after standing

Table 12: Comparison between male (n = 18) and female patients (n = 20) for Ewing's tests

PARAMETER	MEAN VALUE FOR MALES (RANGE)	MEAN VALUES FOR FEMALES (RANGE)	P VALUE
I-E (beats per minute)	22.9 (9.5-35.1)	20.2 (8.2-29.5)	0.240
E:I	1.33 (1.12-1.74)	1.31 (1.12-1.52)	0.690
Valsalva ratio	1.63 (1.26-1.95)	1.76 (1.07-2.39)	0.722
30:15	1.28 (1-1.88)	1.2 (1.0-1.61)	0.260
Diastolic blood pressure change during isometric exercise (mmHg)	15.9 (3.3-30)	9.2 (-6.7-30)	0.013
Systolic blood pressure change during orthostatic load (mmHg)	0.14 (-17.5-18.3)	-0.54 (-15-11.7)	0.807

Abbreviations

I-E Inspiratory-expiratory difference

E:I Ratio of maximum RR interval during expiration to minimum RR interval during inspiration

30:15 Ratio of 30^{th} to 15^{th} RR intervals after standing

Table 13: Mean values for markers of disease severity for male and female patients

PARAMETER	MEAN VALUE FOR MALES (RANGE)	MEAN VALUE FOR FEMALES (RANGE)	P VALUE
Age (yrs)	24.1 (17-41)	23.4 (19-37)	0.724
FEV1 (% predicted)	50.2 (19.7-125)	59.3 (17-88)	0.290
FVC (% predicted)	68.6 (34.4-128)	72.8 (29-92)	0.572
O_2 saturations (%)	95.4 (87-99)	97.1 (95-100)	0.050
Body mass index (kg/m^2)	20.3 (13.9-31.2)	19.8 (14.9-28.8)	0.662
Fasting glucose (mmol/l)	6.5 (3.4-22.4)	6.8 (3.8-24.8)	0.851
HbA1c (%)	6.4 (5.4-10.1)	6.3 (5.1-10.7)	0.770
Vitamin E (umol/l)	13.6 (1-38)	12.7 (2-32)	0.822
No of courses of iv antibiotics over previous 2 yrs	6.7 (1-16)	7.7 (1-21)	0.504
No of days of iv antibiotics over previous 2 yrs	112.8 (7-245)	109.9 (11-351)	0.906

Comparison between diabetic and non-diabetic patients

Patients were classed as diabetic if they were on regular insulin. 13 diabetics and 25 non-diabetics performed Ewing's tests. For each of these tests, there were no significant differences in the mean values between the 2 groups (all cases p=NS) (Table 14). In addition, no differences in mean values for markers of disease severity were observed (Table 15).

Correlations between Ewing's tests and markers of disease severity

Table 16 summarises all significant correlations between markers of disease severity and Ewing's tests for the patient group as a whole, diabetics and non-diabetics. As shown, there were no correlation for the diabetic group.

Table 14: Comparison between diabetic (n = 13) and non-diabetic (n = 25) patients for Ewing's tests

EWING'S TEST	MEAN VALUE FOR DIABETICS (RANGE)	MEAN VALUE FOR NON-DIABETICS (RANGE)	P VALUE
I-E (beats per minute)	24.5 (15.4-32.9)	20.6 (8.2-35.1)	0.092
E:I	1.34 (1.2-1.49)	1.32 (1.12-1.74)	0.677
Valsalva ratio	2.00 (1.68-2.39)	1.48 (1.07-1.95)	0.125
30:15	1.17 (1.0-1.33)	1.28 (1.0-1.88)	0.063
Diastolic blood pressure change during isometric exercise (mmHg)	15 (0-30)	10.3 (-6.7-30)	0.150
Systolic blood pressure change during orthostatic load (mmHg)	-1.9 (-17.5-18.3)	0.4 (-13.3-15)	0.477

Abbreviations

I-E Inspiratory-expiratory difference

E:I Ratio of maximum RR interval during expiration to minimum RR interval during inspiration

30:15 Ratio of 30^{th} to 15^{th} RR intervals after standing

Table 15: Comparison of markers of disease severity between diabetic and non-diabetic patients

PARAMETER	MEAN VALUE FOR DIABETICS (RANGE)	MEAN VALUE FOR NON-DIABETICS (RANGE)	P VALUE
Age (yrs)	22.9 (19-28)	24.2 (17-41)	0.408
FEV1 (% predicted)	52.4 (19.7-88)	56.8 (17-125)	0.620
FVC (% predicted)	74.0 (34.4-106)	69.7 (29-128)	0.565
O2 saturations (%)	96.1 (87-100)	96.5 (92-99)	0.707
Body mass index (kg/m^2)	19.3 (13.9-31.2)	20.5 (14.8-29.4)	0.422
Vitamin E (umol/l)	11 (1-38)	14.2 (2-32)	0.480
No of courses of iv antibiotics over previous 2 yrs	7.62 (1-17)	7 (1-21)	0.710
No of days on iv antibiotics over previous 2 yrs	139.1 (32-351)	96.8 (7-227)	0.162

Table 16: Correlations between Ewing's tests and markers of disease severity

CORRELATION	ALL CF PATIENTS r value	DIABETIC PATIENTS r value	NON-DIABETIC PATIENTS r value
E:I vs FEV1	0.408	No correlation	*0.586
E:I vs FVC	0.342	No correlation	0.444
30:15 vs FEV1	0.348	No correlation	0.487
30:15 vs FVC	No correlation	No correlation	0.478

* $p<0.01$

For all other correlations, $p<0.05$

Summary of results

- The diastolic blood pressure rise during isometric exercise demonstrates the poorest repeatability of all the Ewing's tests.

- Only 7 patients were able to perform the Valsalva manoeuvre. This was not related to any differences in markers of disease severity.

- Significant differences existed between controls and patients for 30:15 ratio, diastolic blood pressure rise on isometric exercise and systolic blood pressure change on standing.

- Microbiological status of the patients did not have any impact on Ewing's tests results.

- Only the diastolic blood pressure change on isometric exercise revealed a significant difference between male and female patients.

- There were no correlations between Ewing's tests results and markers of disease severity in the diabetic group. However, significant relationships were found between parasympathetic test results and pulmonary function for the whole group as well as the subgroup of non-diabetic patients.

3:2 EWING'S TESTS: DISCUSSION

In 1982, Ewing and Clarke adopted a battery of 5 tests, based on 3 parasympathetic and 2 sympathetic tests, to assess cardiovascular autonomic function of any aetiology. Normal, borderline and abnormal ranges were established for each; these are not age-related. Results of these tests (most commonly studied in diabetes mellitus) may be abnormal even before clinical features become apparent.

Control subjects

My data shows that in the control group, the mean values of each of the tests falls within the normal range. Although the majority of controls had results in the normal range, a small proportion were borderline and abnormal. However, in their study of 310 healthy subjects aged 20 to 78 years, O'Brien et al (1986) reported that heart rate responses were less than the 95th centile in at least one of four tests in 12.6% of subjects, and below this limit in two or more tests in 1.6% of subjects (the tests were heart rate variability at rest and in response to a single deep breath, the Valsalva manoeuvre and standing). Ziegler et al (1992) also demonstrated that up to 5% of control subjects had abnormal Ewing's tests results (the lower limit of normal being defined at the 2.3 percentile in that study).

In general, tests of parasympathetic function are more reproducible than tests of sympathetic function; Hague et al (1978) showed that the blood pressure response to sustained handgrip and fall in blood pressure on standing were less reliable than tests of heart rate change. Piha et al (1991) conducted a repeatability and reproducibility study in normal control subjects aged 21 to 56 years. Using 3 measurements within a 24 hour period, mean values for coefficients of variation for the expiratory:inspiratory (E:I) ratio, Valsalva ratio and 30:15 ratio were 4%, 7% and 7% respectively. This was virtually unchanged over a 3 week and 2 year period. Risk et al (2001) performed a repeatability study (3 times within a day) on 45 healthy subjects (18 women, mean age 37$\pm$15 years; 27 men, mean age 45$\pm$16 years). Mean coefficients of variation for E:I ratio, Valsalva ratio and 30:15 ratio

were 4.3%, 6.26% and 6.66% respectively. These results are comparable to my values of 5.44%, 8.10% and 6.75%.

In another study of 10 healthy subjects, carried out on 5 successive days at the same time each day, the mean standard deviation of the inspiratory-expiratory (I-E) difference during 6 cycles of deep breathing was 4.88 beats per minute; a coefficient of variation was not reported. My repeatability study shows the mean standard deviation of I-E was 3.58 beats per minute.

When looking at tests of sympathetic function, the values of coefficients of variation are much higher. Piha et al (1991) reported that the mean coefficient of variation of increase in diastolic blood pressure in response to isometric exercise was 34% (the method of blood pressure measurement was not indicated in this study). My value of 69.62% is higher. The mean standard deviation is also higher (8.74mmHg) compared to Comi et al (1986) (6.1mmHg). This reflects a wide intra-individual variation in diastolic blood pressure change. It is possible that blood pressure measurement in my study may have been more accurate had I used the non-invasive photoplethysmographic Finapres device. However, there is some controversy regarding the accuracy of this device when compared to the gold standard of intra-arterial blood pressure measurement. For example, Kermode et al (1989) compared the Finapres device with intra-arterial pressure monitoring in 20 patients during induction of anaesthesia for elective neurosurgical procedures. The differences between the two methods were considerable, ranging from –40mmHg to +26mmHg for mean pressure; the Finapres displayed too great a variability for it to be used as an alternative to intra-arterial pressure monitoring. In addition, Idema et al (1989) evaluated the use of Finapres blood pressures in 6 normotensive healthy males during increasing levels of bicycle exercise using simultaneously registered ipsilateral intrabrachial artery pressures as a reference. At rest, finger systolic blood pressure was higher and finger diastolic and mean arterial pressures were lower than the corresponding intrabrachial pressures in 5 of the 6 subjects. During exercise, average finger diastolic and mean arterial pressures did not differ further from the intrabrachial pressure, but finger systolic pressure increased considerably more than the direct systolic pressure, exceeding it by 26$\pm$20mmHg at maximal exercise. Furthermore, my protocol for handgrip exercise involved squeezing a blood pressure cuff; it is possible that some subjects varied the amount

of pressure exerted through their fingers to make it easier to cope with the stress associated with the test.

The value of 3.7mmHg for change in systolic blood pressure on standing in the study by Comi et al (1986) is higher than 1.22 mmHg from our data. This could again be a reflection of differences in blood pressure measurement. Overall, the data from the literature and my work indicates the superiority of tests of parasympathetic function.

Test results of autonomic function are said to decline with age. Wieling et al (1982) studied parasympathetic function tests in 133 healthy subjects. The lower limit of normal decreased from 22 to 11 beats per minute for deep breathing and from 26 to 11 beats per minute for standing up as age increased from 10 to 65 years. Smith (1982) has also demonstrated reduced sinus arrhythmia with age in diabetic autonomic neuropathy. van Dijk et al (1991) showed that a relationship with age existed for all parasympathetic tests but blood pressure responses were not significantly related to age. My data indicates that there was, at best, a weakly negative correlation between inspiratory-expiratory difference and systolic blood pressure change during orthostatic load and age ($r=-0.659$, $p<0.05$ and $r=-0.689$, $p<0.05$ respectively). This can be explained by all control subjects being less than 40 years old.

Patients

38 patients participated in the study, but only 7 were able to complete the Valsalva manoeuvre. This, however, was not related to differences in pulmonary function or any other marker of disease severity.

Spirometric indices and body mass index are the most commonly used markers of disease severity (Lees and Smyth, 2000). Glucose and HbA1c levels are indicators of diabetic control which can be associated with autonomic neuropathy. Vitamin E deficiency can result in neurological abnormalities in CF patients (Sitrin et al, 1987) and although the presence of autonomic neuropathy has not previously been investigated in this patient group, vitamin E administration to diabetic patients has been shown to increase the RR interval and total autonomic function (Manzella et

al, 2001). Finally, the number of courses and days of intravenous antibiotics could also be a reflection of disease progression. For my patient group, only the mean values for fasting glucose and vitamin E levels were outside the normal range (where applicable).

Mean values for all of Ewing's tests were within the normal range. This could be a reflection of the young age of most of my patients. It could also indicate a lack of sensitivity of Ewing's tests particularly sympathetic function, as supported by Low et al (1986) who demonstrated a higher frequency of vagal dysfunction compared to orthostatic hypotension in diabetics with neuropathy.

Other criticisms of Ewing's tests are that they require active patient participation which is difficult to standardise. Several different methods of quantification and protocols exist which makes comparison between studies difficult. For example, Ryder and Hardisty (1990) have claimed that heart rate changes evoked by a single deep breath are greater than those evoked by repeated breaths. However, Espi et al (1982) reported the first deep breath consistently produced the largest heart rate variation in only 29% of normal subjects and 17% of diabetics and that between-test variability was smaller with repeated deep breaths. In addition, it is said that the expiratory:inspiratory ratio is significantly related to resting heart rate in contrast to heart rate variability evaluated by differences between maximum and minimum heart rate (van Dijk et al, 1991).

Use of the 30:15 ratio has also been criticised. Ewing and Clarke (1982) originally described this as the ratio of the longest RR interval at around the 30^{th} beat after starting to stand to the shortest RR interval at around the 15^{th} beat. However, Ziegler et al (1992) found that the longest RR interval around beat 30 occurred within beats 21 to 40 while the shortest RR interval around beat 15 occurred within beats 6 to 24.

Low et al (1975) described some spurious results with the Valsalva manoeuvre as without invasive beat-to-beat blood pressure recordings, only the compensatory heart rate change was being recorded. O'Brien et al (1986) claimed that one attempt at the Valsalva manoeuvre was sufficient but Ewing (1990) reported that patients could perform this incorrectly and therefore three attempts should be made.

The diastolic blood pressure rise during isometric exercise, due to its limited repeatability, is not suitable for assessment of decline of autonomic function over

time, and finally both this and the systolic blood pressure response to standing depend on resting blood pressure (van Dijk et al, 1991). They are difficult to measure accurately unless invasive intra-arterial monitoring is used. Although my data indicated the greatest percentage of abnormal results (50%) for isometric exercise in the patient group, this should be interpreted with caution for the reasons outlined above.

Once again, in my study, only a test of heart rate change (E:I) negatively correlated with age ($r=-0.447$, $p<0.01$), again reflecting the young age of the patients.

Without taking the isometric exercise response into account due to poor reproducibility in controls, more patients had abnormal parasympathetic results than for example systolic blood pressure change on standing. This could reflect the sequence of changes in autonomic neuropathy. Ewing et al (1985) examined 237 subjects whose cardiovascular tests were repeated at least 3 months apart; the results of 71% were unchanged, 26% deteriorated and 3% improved. First heart rate and then blood pressure abnormalities developed. Toyry et al (1996) studied 133 diabetics at baseline, 5 and 10 years later. The frequency of parasympathetic damage was 4.9% at baseline, 19.6% versus 6.8% for sympathetic damage at 5 years and 65% versus 9% at 10 years. These studies show that parasympathetic dysfunction precedes sympathetic damage.

Comparison with controls

Only mean values for the 30:15 ratio and systolic blood pressure change on standing were significantly different between the 2 groups. This is not unexpected as both these tests involve the same baroreceptor reflex arc.

The p value of 0.02 for difference in diastolic blood pressure change on isometric exercise should be viewed with caution on account of its poor repeatability.

Sundkvist et al (1979) found that the E:I ratio was significantly different in diabetics compared with controls. However, patient selection may be criticised as both controls and diabetic subjects included smokers. This could result in accelerated coronary artery disease which could affect cardiac function and so the differences would not be valid.

In my study, no significant differences existed between the 2 groups for I-E possibly reflecting the wide variation in individual results particularly in the patients (mean standard deviation 7.08 beats per minute). Again E:I did not differ between the 2 groups, although this ratio is influenced by resting heart rate.

Values for the Valsalva ratio also exhibited wide variation in the patient group (standard deviation 0.445). This is in contrast to the work of Ziegler et al (1992) who demonstrated that the Valsalva ratio discriminated well between controls and diabetics (17.5% abnormal for diabetics versus 4.2% abnormal for controls, $p<0.001$). Had more patients been able to complete the Valsalva manoeuvre in my study (n=7), then perhaps the difference with controls might have been more significant.

Effect of microbiological status

Colonisation of CF patients with *Burkholderia cepacia* results in greater morbidity and impairment of pulmonary function compared to *Pseudomonas* colonised patients; a syndrome characterised by high fevers, severe progressive respiratory failure, leucocytosis and elevated inflammatory markers can occur with a high fatality rate (Isles et al, 1984). Furthermore, epidemic strains are capable of cross-colonising CF patients already infected with *B cepacia*, often with fatal consequences (Ledson et al, 1998). I therefore investigated the possibility that *B cepacia* colonised patients might have worse autonomic function on the basis of Ewing's tests. However, this was not the case, possibly reflecting lack of sensitivity of Ewing's tests.

Comparison between male and female patients

The only significant difference between male and female patients was for the sustained handgrip test (mean values males 15.9mmHg versus females 9.2mmHg, $p<0.013$). Gender differences in the response to isometric exercise have previously been reported. Piha (1993) examined the results of cardiovascular autonomic tests on 224 healthy subjects. The diastolic blood pressure response to isometric exercise was higher in males under 50 years than in females of the same

age ($p<0.05$). This was confirmed by Khurana and Setty (1996) in a study of control and dysautonomic subjects.

Comparison between diabetic and non-diabetic patients

52% of the patient group was diabetic (those requiring regular insulin). As diabetes contributes towards autonomic neuropathy, it was expected that significant differences in autonomic function might exist between the 2 groups. This was not the case; nor were there any differences in markers of disease severity.

However, CF-related diabetes mellitus (CFRDM) differs from conventional Type I and Type II diabetes. Although in CF the age of onset of CFRDM is 15 to 25 years, it is unlike Type I in that it is of slow onset (prodromal period often 2 years or more), there is persistence of some insulin secretion, ketosis is not a feature and autoimmune factors are rarely present. It is not associated with obesity (patients are often underweight) as in Type II diabetes. There may be insulin deficiency as opposed to hyperinsulinaemia and insulin resistance. There is also an associated pancreatic exocrine defect (Hodson, 1992).

Correlations with markers of disease severity

For the patient group as a whole, only the E:I and 30:15 ratios correlated with FEV1 and FVC% predicted. The former tests had the best repeatability. It is disappointing that there were no relationships with any other markers of disease severity, however pulmonary function is considered to be an accurate marker of disease severity; Kerem et al (1992) showed that a fall in FEV1 to less than 30% predicted was associated with a 50% risk of death within 2 years.

No correlations were found in the diabetic group but as explained above CFRDM is an entity distinct from conventional diabetes.

For non-diabetic patients, there were also significant correlations between E:I and 30:15 ratios and spirometric indices. Since there were no differences in markers of disease severity between diabetics and non-diabetics and no correlations in diabetics with any markers of disease severity, a mechanism which does not

involve the effects of hyperglycaemia, nutritional state, vitamin E deficiency and number of courses and days of intravenous antibiotics could be responsible for autonomic neuropathy in CF.

CHAPTER 4: CARDIOVASCULAR SYSTEM (II)

4:1 SPECTRAL ANALYSIS: RESULTS

4:2 SPECTRAL ANALYSIS: DISCUSSION

4:1 SPECTRAL ANALYSIS: RESULTS

Control subjects

38 control subjects participated in the study; 6 male and 32 female. The mean age was 30 years (range 22-39 years). All subjects completed the full 15 minute modified orthostatic load.

Table 1 shows the mean value and range for each parameter of spectral analysis. There were no significant correlations with age.

In order to establish the repeatability of each parameter, measurements were made on 3 control subjects on 3 successive days. The data was then subjected to a logarithmic transformation and using the method of Ziegler et al (1992) the standard deviation factor (SDF_{intra}) was calculated as follows:

SDF_{intra} = antilog of the square root of the mean of the variances resulting from each triad of log-transformed values

The coefficient of variation of the RR interval was 6.33%. Table 2 indicates the SDF_{intra} for the remaining parameters. These values are comparable to those in the literature (see Discussion on spectral analysis). Appendix D shows the data for individual controls.

To demonstrate the recovery of the autonomic nervous system back to the initial resting state, spectral analysis parameters in both the initial and final supine positions were compared. Table 3 shows there were no significant differences.

Effect of bronchodilators

It was recognised that nebulised β-agonists could have a potentially stimulatory effect on sympathetic function (low frequency power) and heart rate. To address this further, 5mg nebulised salbutamol was administered to a control subject and spectral analysis parameters measured at intervals over a 3 hour period. Figs 1 and 2 illustrate that the effects had worn off by 30 minutes.

Table 1: Mean values for each parameter of spectral analysis in the control group

PARAMETER (initial supine position)	MEAN VALUE (RANGE)
Total power (ms^2)	1669.33 (196.93-8168.33)
High frequency power (ms^2)	1153.35 (29.56-7287.55)
Low frequency power (ms^2)	516.01 (69.11-2676.13)
Low to high frequency ratio (sympathovagal balance)	0.93 (0.02-5.80)
RR interval length (sec)	0.888 (0.620-1.254)
Cumulative power (global autonomic tone) (ms^2)	4484.97 (624.14-170428.80)

Table 2: Repeatability study for spectral analysis after logarithmic transformation of the data

PARAMETER (initial supine position)	STANDARD DEVIATION FACTOR
Total power	1.396
High frequency power	1.546
Low frequency power	1.513
Cumulative power	1.270

Table 3: Comparison of spectral analysis parameters between initial and final supine positions

PARAMETER	MEAN VALUE FOR INITIAL POSITION (RANGE)	MEAN VALUE FOR FINAL POSITION (RANGE)	P VALUE
Total power (ms^2)	1669.33 (196.93-8168.33)	1989.46 (201.69-11319.0)	0.52
High frequency power (ms^2)	1153.35 (29.56-7287.55)	1524.07 (35.74-10466.0)	0.40
Low frequency power (ms^2)	516.01 (69.12-2676.13)	465.39 (76.37-2433.93)	0.67
Low to high frequeny ratio	0.934 (0.022-5.796)	0.785 (0.034-4.635)	0.51
RR interval length (sec)	0.888 (0.620-1.254)	0.928 (0.647-1.215)	0.143

Fig 1: **Evolution of sympathetic function over time after administration of nebulised salbutamol to a control subject**

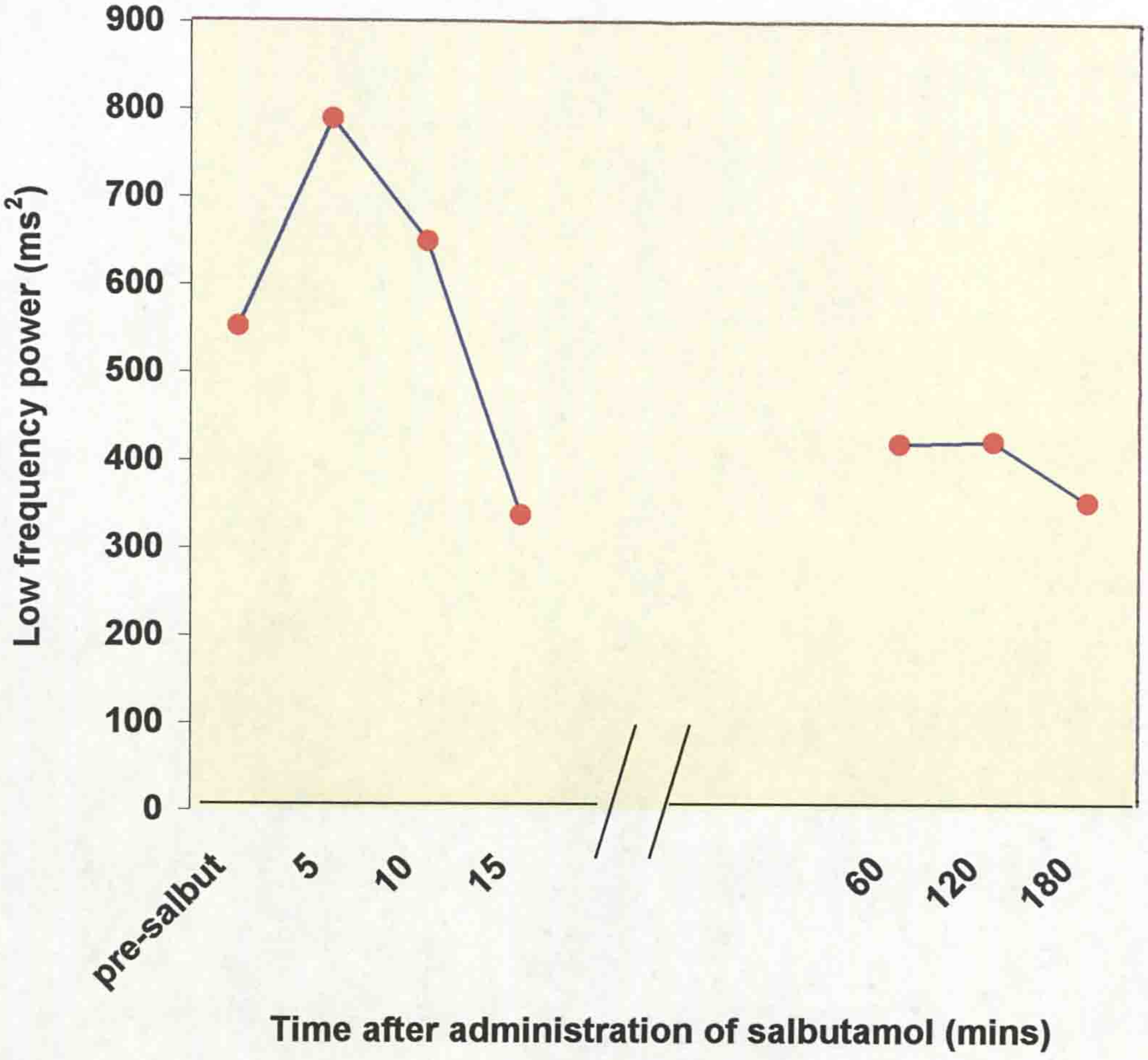
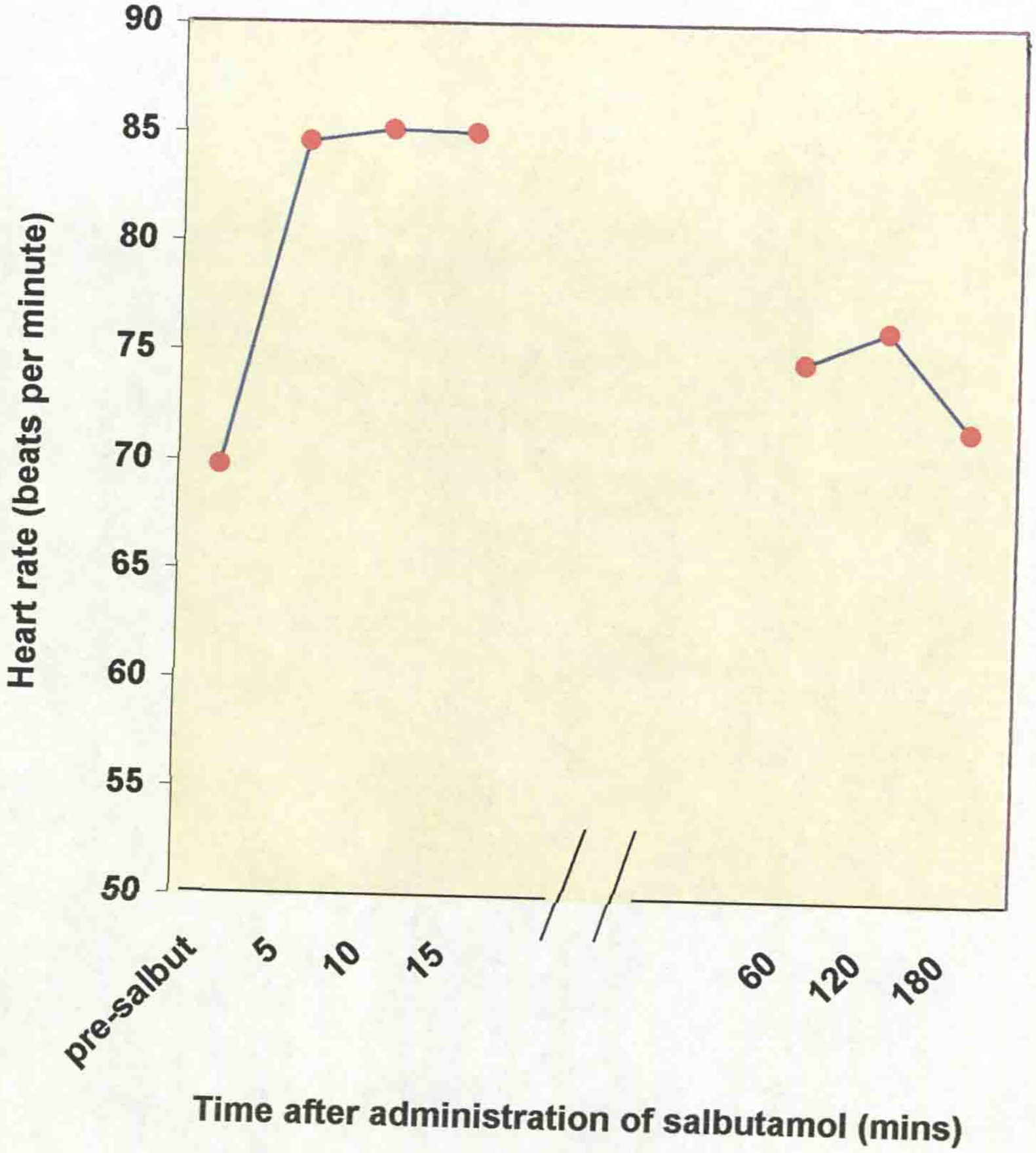


Fig 2: **Evolution of heart rate over time after administration of nebulised salbutamol to a control subject**



Patients' results

49 patients were included, 23 male and 26 female. The mean age was 24.7 years (range 17-42 years). All patients were in a stable clinical state and tested at the same time each day.

As for Ewing's tests, the following markers of disease severity were determined: spirometric indices, body mass index, fasting glucose, HbA1c, vitamin E levels, number of courses of intravenous antibiotics and number of days spent on intravenous antibiotics over the preceding 2 years. Mean values and ranges are shown in Table 4.

Table 5 indicates the mean values and ranges for spectral analysis parameters.

The proportion of patients with abnormal results is given in Table 6. As there are no well-defined ranges for spectral analysis parameters, a result was defined as abnormal if it was below the value for the 5^{th} centile calculated from the group of control subjects.

As in the controls, the recovery of the autonomic nervous system back to its initial resting state was demonstrated by comparing mean values for spectral analysis parameters in the initial and final supine states (Table 7). Once again, there were no significant differences.

Table 4: Mean values for markers of disease severity in the patient group

PARAMETER	MEAN VALUE (RANGE)
FEV1 (% predicted)	54.5 (17-125)
FVC (% predicted)	71.2 (29-128)
O2 saturation (%)	96.3 (87-100)
Body mass index (kg/m^2)	20.4 (13.9-31.4)
Fasting glucose (mmol/l)	6.7 (3.4-24.8)
HbA1C (%)	6.3 (5.1-10.7)
Vitamin E (umol/l)	15.6 (1-38)
No of courses of iv antibiotics over previous 2 yrs	7.0 (1-21)
No of days on iv antibiotics over previous 2 yrs	112.6 (7-511)

Table 5: Mean value for spectral analysis parameters in the patient group

PARAMETER	MEAN VALUE (RANGE)
Total power (ms^2)	714.18 (30.11-4996.43)
High frequency power (ms^2)	339.23 (1.95-2622.5)
Low frequency power (ms^2)	374.95 (21.83-2374)
Low to high frequency power (sympathovagal balance)	2.90 (0.089-14.33)
RR interval length (sec)	0.683 (0.509-0.937)
Cumulative power (global autonomic tone) (ms^2)	1992.36 (133.61-10270.0)

Table 6: The proportion of patients with abnormal spectral analysis parameters

PARAMETER	5^{th} CENTILE (CONTROL SUBJECTS)	PROPORTION WITH ABNORMAL RESULTS ($<5^{th}$ centile) (%)
Total power	197.09 ms^2	10/49 (20.4)
High frequency power	67.38 ms^2	15/49 (30.6)
Low frequency power	70.39 ms^2	6/49 (12.2)
RR interval length	0.631 sec	12/40 (30)
Cumulative power	679.895 ms^2	12/48 (25)

Due to computer failure, values for RR intervals and cumulative power were available for only 40 and 48 patients respectively.

Table 7: Comparison between spectral analysis parameters in the initial and final states for patients

PARAMETER	INITIAL SUPINE STATE (RANGE)	FINAL SUPINE STATE (RANGE)	P VALUE
Total power (ms^2)	714.18 (30.11-4996.54)	948.05 (49.22-4077.50)	0.22
High frequency power (ms^2)	339.23 (1.95-2622.50)	492.94 (4.98-3735.30)	0.24
Low frequency power (ms^2)	374.95 (21.83-2374.0)	455.11 (29.84-2162.30)	0.38
Low to high frequency ratio (sympathovagal balance)	2.903 (0.089-14.331)	3.563 (0.092-38.194)	0.52
RR interval length (sec)	0.683 (0.509-0.937)	0.694 (0.520-1.015)	0.639

Comparison with controls

As demonstrated in Table 8, when compared to the control group, the CF patients had significantly worse values for all spectral analysis parameters except low frequency power (controls mean: 516.0ms^2, patients mean: 374.95ms^2, p=NS). This could not be explained by the effects of β-agonists (see earlier in the section on 'Effects of bronchodilators'). In addition, a comparison of spectral parameters between those patients receiving regular nebulised salbutamol (n=43) and those not taking this treatment (n=6) revealed no significant differences (Table 9).

Comparison between patients colonised with *Burkholderia cepacia* and *Pseudomonas aeruginosa*

34 patients (mean age 23.5 years, range 17-42 years) were colonised with *Pseudomonas aeruginosa*. 14 patients (mean age 25.7 years, range 19-39 years) were colonised with *Burkholderia cepacia* (one was not colonised with either organism). There were significant differences in spectral analysis parameters between these 2 groups (Table 10), but this was not accounted for by any significant differences in markers of disease severity (Table 11).

Comparison between male and female patients

Tables 12 and 13 illustrate the differences between male and female patients for spectral analysis parameters and markers of disease severity respectively. In all cases, p=NS.

Table 8: Comparison of spectral analysis parameters between patients and control subjects

PARAMETER	MEAN VALUE FOR CONTROLS (RANGE)	MEAN VALUE FOR PATIENTS (RANGE)	P VALUE
Total power (ms^2)	1669.33 (196.93-8168.33)	714.18 (30.11-4996.43)	0.004
High frequency power (ms^2)	1153.35 (29.56-7287.55)	339.23 (1.95-2622.5)	0.004
Low frequency power (ms^2)	516.01 (69.11-2676.13)	374.95 (21.83-2374)	0.20
Low to high frequency ratio (sympathovagal balance)	0.93 (0.02-5.80)	2.90 (0.089-14.33)	< 0.0001
RR interval (sec)	0.888 (0.620-1.254)	0.683 (0.509-0.937)	< 0.0001
Cumulative power (ms^2)	4484.97 (624.14-170428.80)	1992.36 (133.61-10270.0)	0.003

Table 9: Comparison of spectral analysis parameters between those patients who were on regular nebulised bronchodilators (n=43) and those who were not (n=6)

PARAMETER	MEAN VALUE FOR PATIENTS ON NEBULISED BRONCHO-DILATORS (RANGE)	MEAN VALUE FOR PATIENTS NOT ON NEBULISED BRONCHO-DILATORS (RANGE)	P VALUE
Total power (ms^2)	752.08 (30.1-4996.54)	442.55 (46.05-1064.84)	0.173
High frequency power (ms^2)	360.57 (1.95-2622.5)	186.28 (7.47-760.181)	0.247
Low frequency power (ms^2)	391.52 (21.83-2374.0)	256.26 (38.47-641.46)	0.231
Low to high frequency ratio	2.812 (0.089-14.331)	3.559 (0.406-5.447)	0.443
RR interval length (sec)	0.690 (0.509-0.937)	0.645 (0.539-0.709)	0.173
Cumulative power (ms^2)	2088.64 (133.61-10270)	1318.41 (145.01-3860.5)	0.262

Table 10: Comparison of spectral analysis parameters between patients colonised with *Pseudomonas aeruginosa* (n=34) and *Burkholderia cepacia* (n=14)

PARAMETER	MEAN VALUE FOR *Psa* PATIENTS (RANGE)	MEAN VALUE FOR *B cepacia* PATIENTS (RANGE)	P VALUE
Total power (ms^2)	874.37 (46.05-4996.54)	352.33 (30.11-1270.25)	0.006
High frequency power (ms^2)	421.09 (7.48-2622.5)	151.98 (1.94-532.28)	0.016
Low frequency power (ms^2)	453.28 (22.37-2374.0)	200.35 (21.828-737.98)	0.009
Low to high frequency ratio	2.440 (0.089-10.751)	4.173 (0.208-14.331)	0.181
RR interval length (sec)	0.69 (0.509-0.937)	0.657 (0.538-0.855)	0.184
Cumulative power (ms^2)	2343.59 (145.01-10270)	1144.899 (133.61-5064.5)	0.026

Table 11: Comparison of markers of disease severity between patients colonised with *Pseudomonas aeruginosa* and *Burkholderia cepacia*

PARAMETER	MEAN VALUE FOR *Psa* PATIENTS (RANGE)	MEAN VALUE FOR *B cepacia* PATIENTS (RANGE)	P VALUE
Age (yrs)	23.5 (17-42)	25.7 (19-39)	0.239
FEV1 (% predicted)	57.8 (24-125)	44.1 (17-88)	0.100
FVC (% predicted)	75.1 (45-128)	60.1 (29-93)	0.065
O2 saturations (%)	96.9 (93-100)	94.5 (87-97)	0.037
Body mass index (kg/m^2)	20.1 (13.9-29.4)	20.4 (14.7-31.2)	0.781
Fasting glucose (mmol/l)	6.6 (3.8-22.4)	6.8 (58-128)	0.915
HbA1c (%)	6.2 (5.1-10.1)	6.5 (5.5-10.7)	0.542
Vitamin E (umol/l)	15.8 (2-38)	13.8 (1-32)	0.612
No of courses of iv antibiotics over previous 2 yrs	6.7 (1-21)	8.1 (1-14)	0.299
No of days on iv antibiotics over previous 2 yrs	107.7 (7-511)	131.6 (27-238)	0.356

Table 12: Comparison of spectral analysis parameters between male (n=23) and female (n=26) patients

PARAMETER	MEAN VALUE FOR MALES (RANGE)	MEAN VALUE FOR FEMALES (RANGE)	P VALUE
Total power (ms^2)	716.12 (46.04-4996.54)	712.47 (30.1-2093.23)	0.988
High frequency power (ms^2)	315.02 (7.48-2622.5)	360.64 (1.95-1565.47)	0.752
Low frequency power (ms^2)	401.09 (38.47-2374.0)	351.83 (21.83-1169.9)	0.685
Low to high frequency ratio	2.768 (0.456-7.648)	3.023 (0.089-14.331)	0.769
RR interval length (sec)	0.686 (0.509-0.937)	0.681 (0.518-0.793)	0.873
Cumulative power (ms^2)	1936.56 (145.01-10270)	2039.57 (133.61-6574.1)	0.865

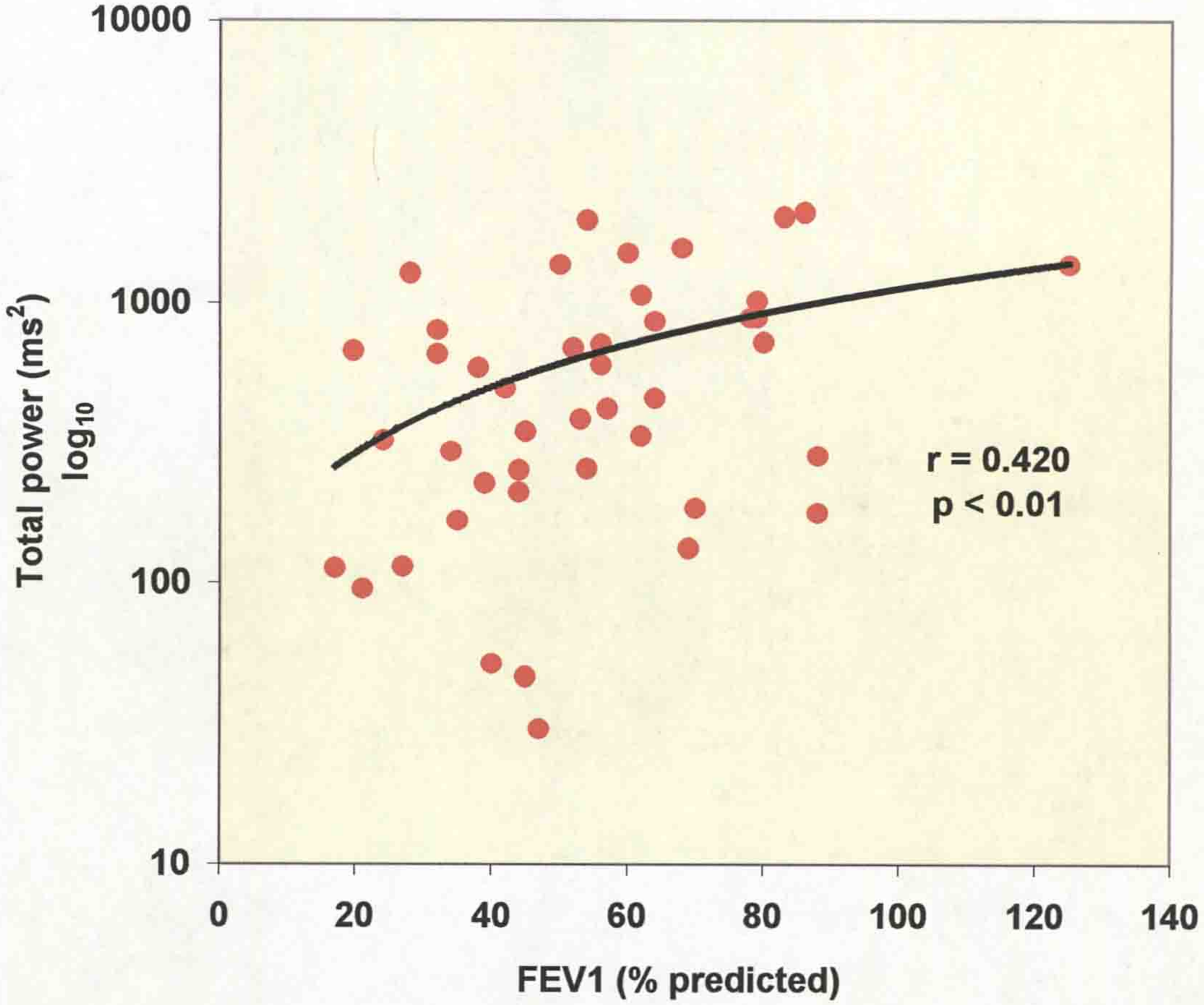
Table 13: Comparison of markers of disease severity between male and female patients

PARAMETER	MEAN VALUE FOR MALES (RANGE)	MEAN VALUE FOR FEMALES (RANGE)	P VALUE
Age (yrs)	24.1 (17-41)	24.2 (18-42)	0.989
FEV1 (% predicted)	52.5 (19.7-125)	55.6 (17-88)	0.675
FVC (% predicted)	69.36 (34.4-128)	72.8 (29-101)	0.594
O2 saturations (%)	95.5 (87-99)	96.8 (93-100)	0.080
Body mass index (kg/m^2)	20.4 (13.9-31.2)	20.4 (14.9-31.4)	0.949
Fasting glucose (mmol/l)	6.5 (3.4-22.4)	7.0 (3.8-24.8)	0.670
HbA1C (%)	6.3 (5.4-10.1)	6.25 (5.1-10.7)	0.9
Vitamin E (umol/l)	15.2 (1-38)	15.4 (2-37)	0.95
No of courses of iv antibiotics over previous 2 yrs	6.3 (1-16)	7.54 (1-21)	0.370
No of days on iv antibiotics over previous 2 yrs	121.1 (7-511)	104.5 (11-351)	0.555

Correlations between spectral analysis parameters and markers of disease severity in the whole patient group

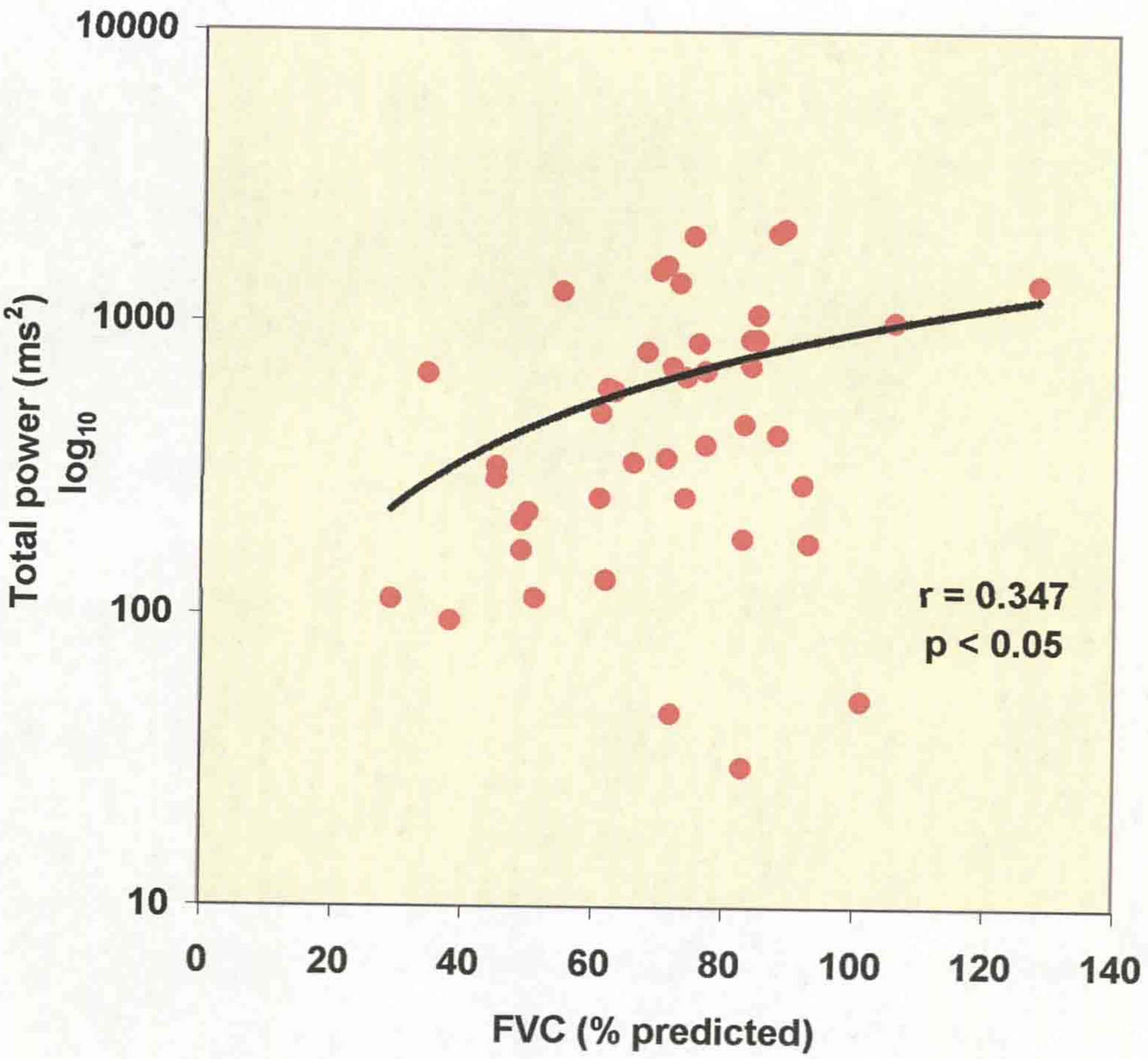
Significant correlations existed between all spectral parameters and spirometric indices, except for the low to high frequency ratio (an index of sympathovagal balance) which correlated with fasting glucose ($p<0.01$) and HbA1c ($p<0.001$). This is demonstrated in Figs 3 to 12.

Fig 3: Correlation between total power and lung function (FEV1% predicted) in 44 patients



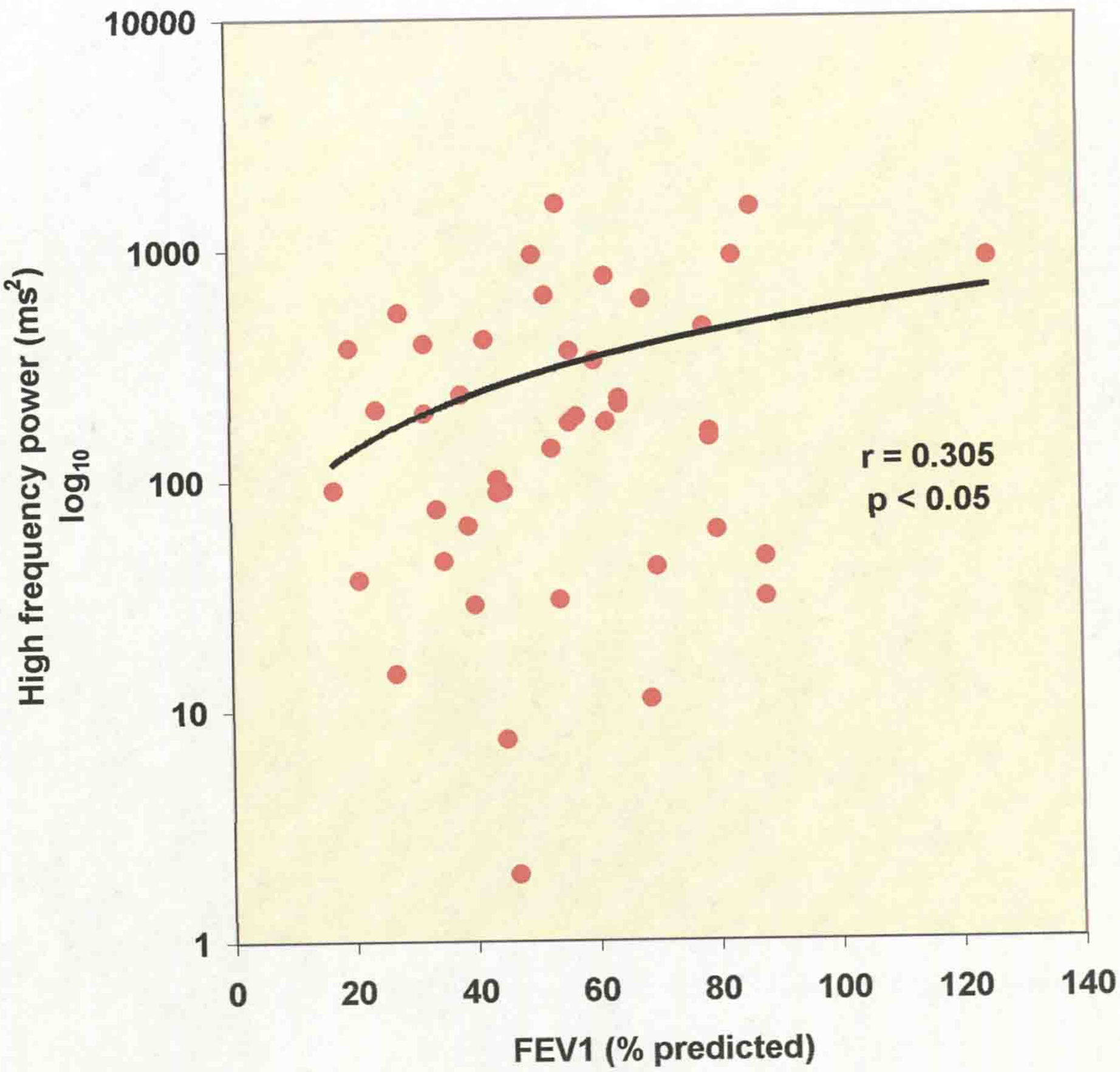
FEV1 (% predicted) was available for only 44 patients as not all of the original group (n=49) were able to perform spirometry

Fig 4: Correlation between total power and lung function (FVC% predicted) in 44 patients



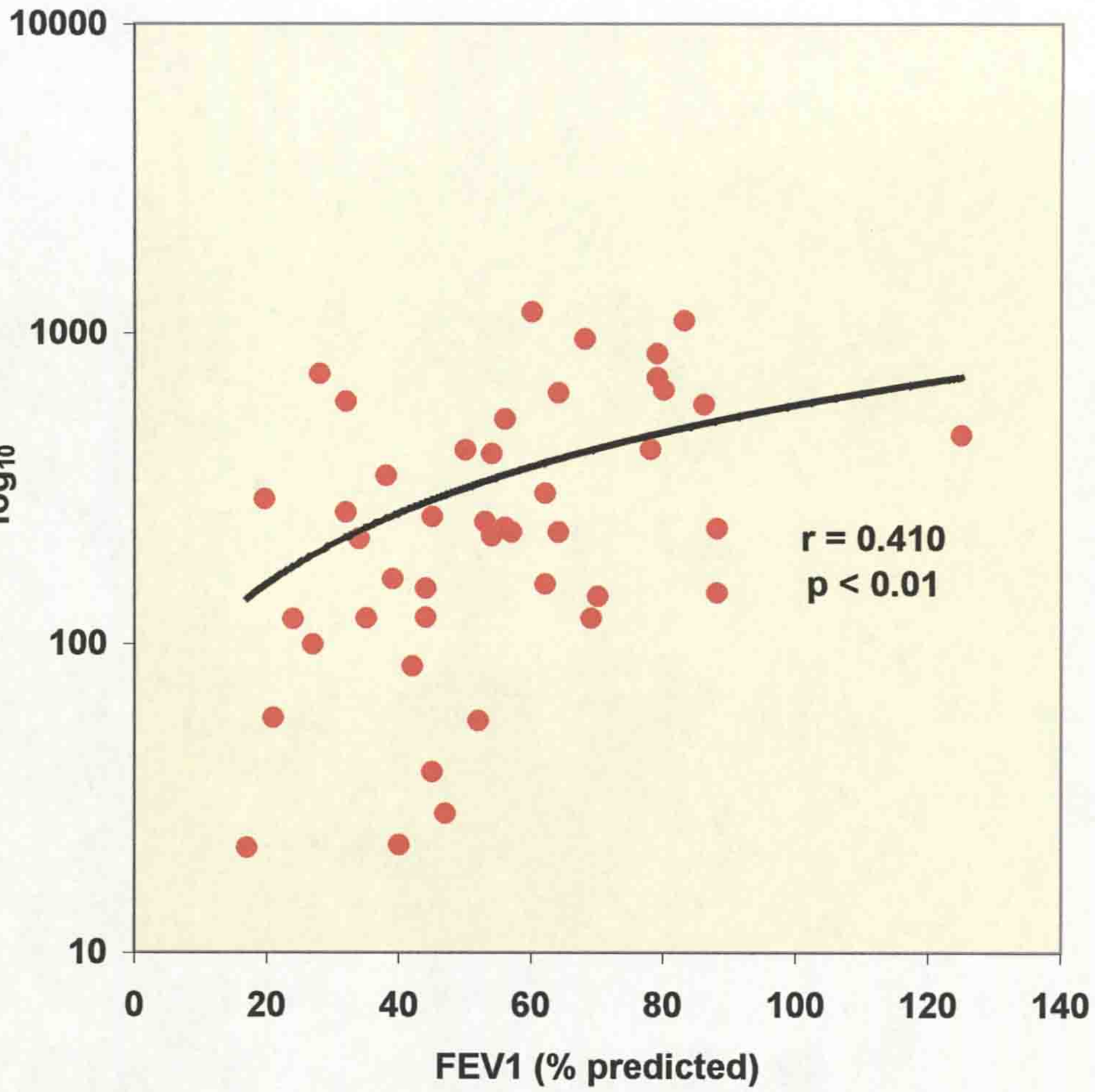
FEV1 (% predicted) was available for only 44 patients as not all of the original group (n=49) were able to perform spirometry.

Fig 5: Correlation between parasympathetic function and lung function (FEV1% predicted) in 44 patients



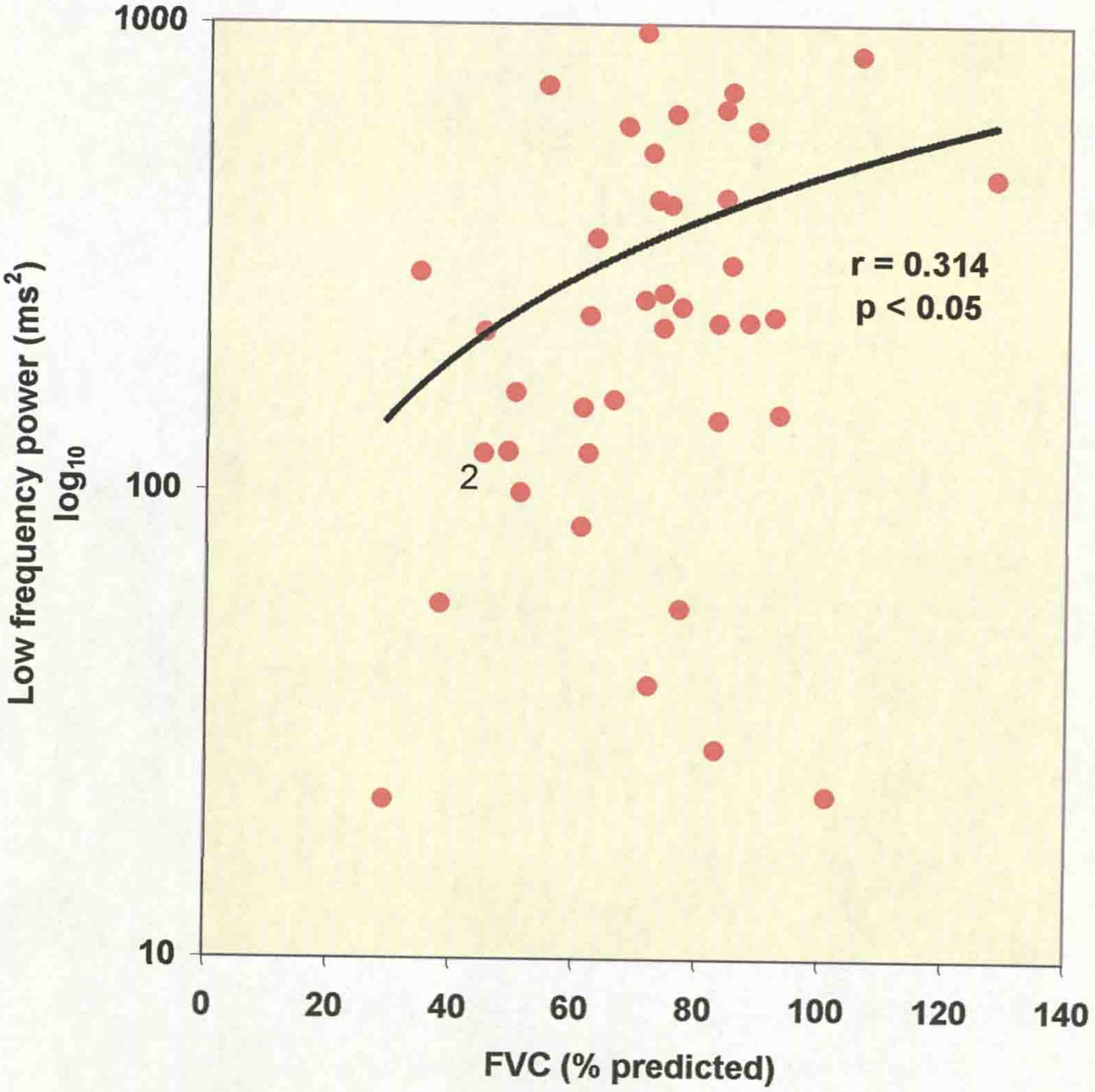
FEV1 (% predicted) was available for only 44 patients as not all of the original group (n=49) were able to perform spirometry.

Fig 6: Correlation between sympathetic function and lung function (FEV1% predicted) in 44 patients



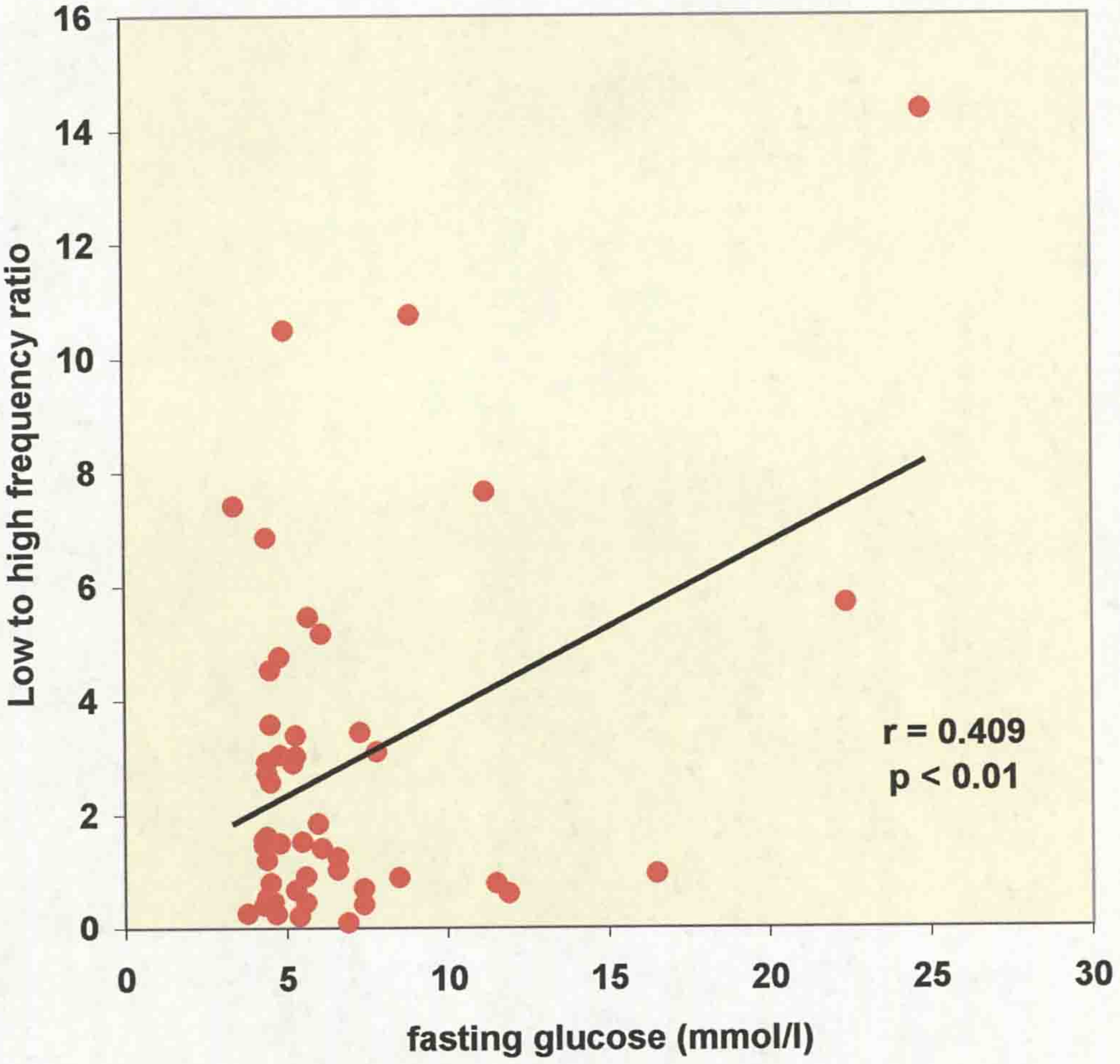
FEV1 (% predicted) was available for only 44 patients as not all of the original group (n=49) were able to perform spirometry.

Fig 7: Correlation between sympathetic function and lung function (FVC% predicted) in 44 patients



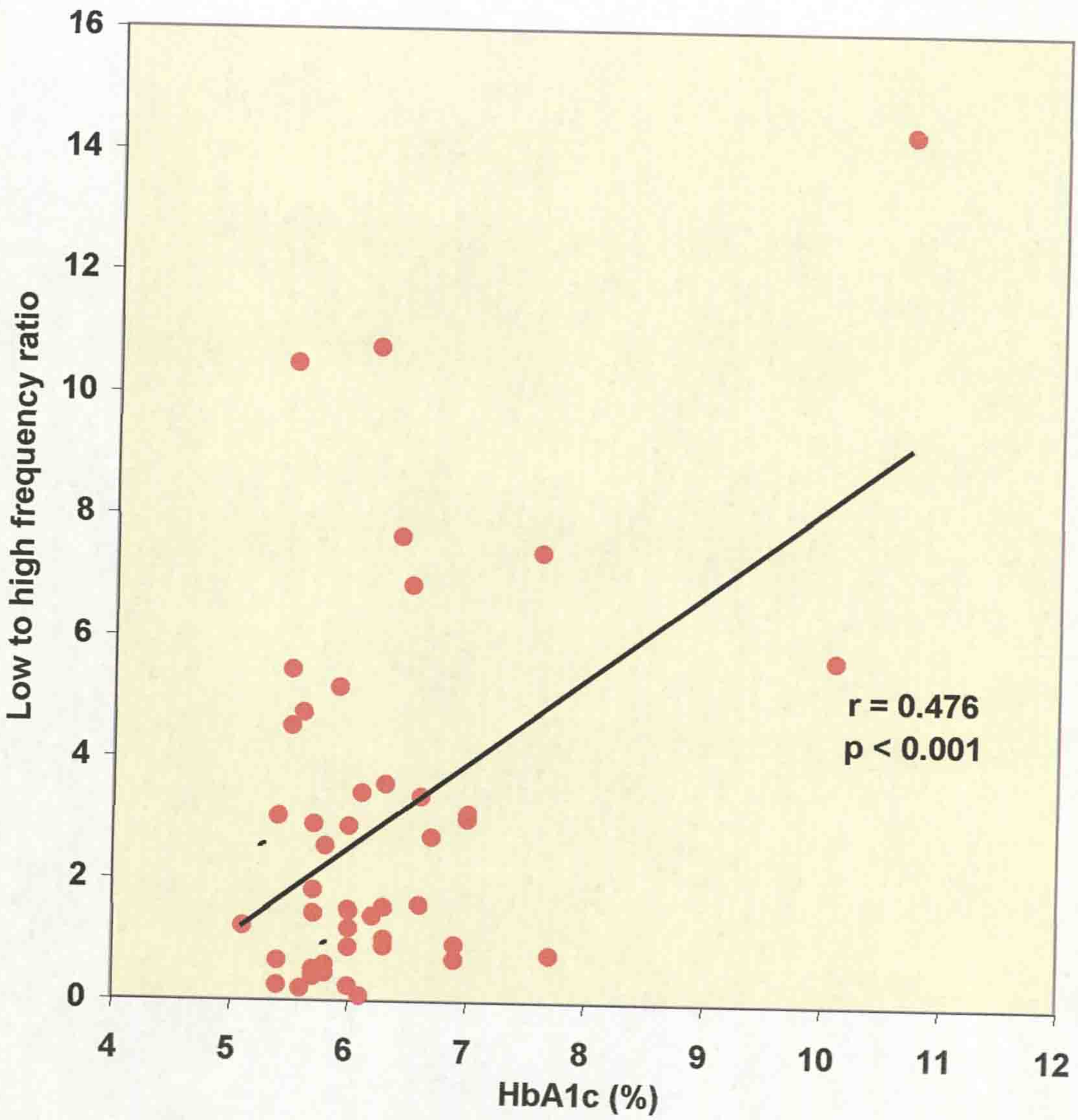
FEV1 (% predicted) was available for only 44 patients as not all of the original group (n=49) were able to perform spirometry.

Fig 8: Correlation between sympathovagal balance and fasting glucose in 48 patients



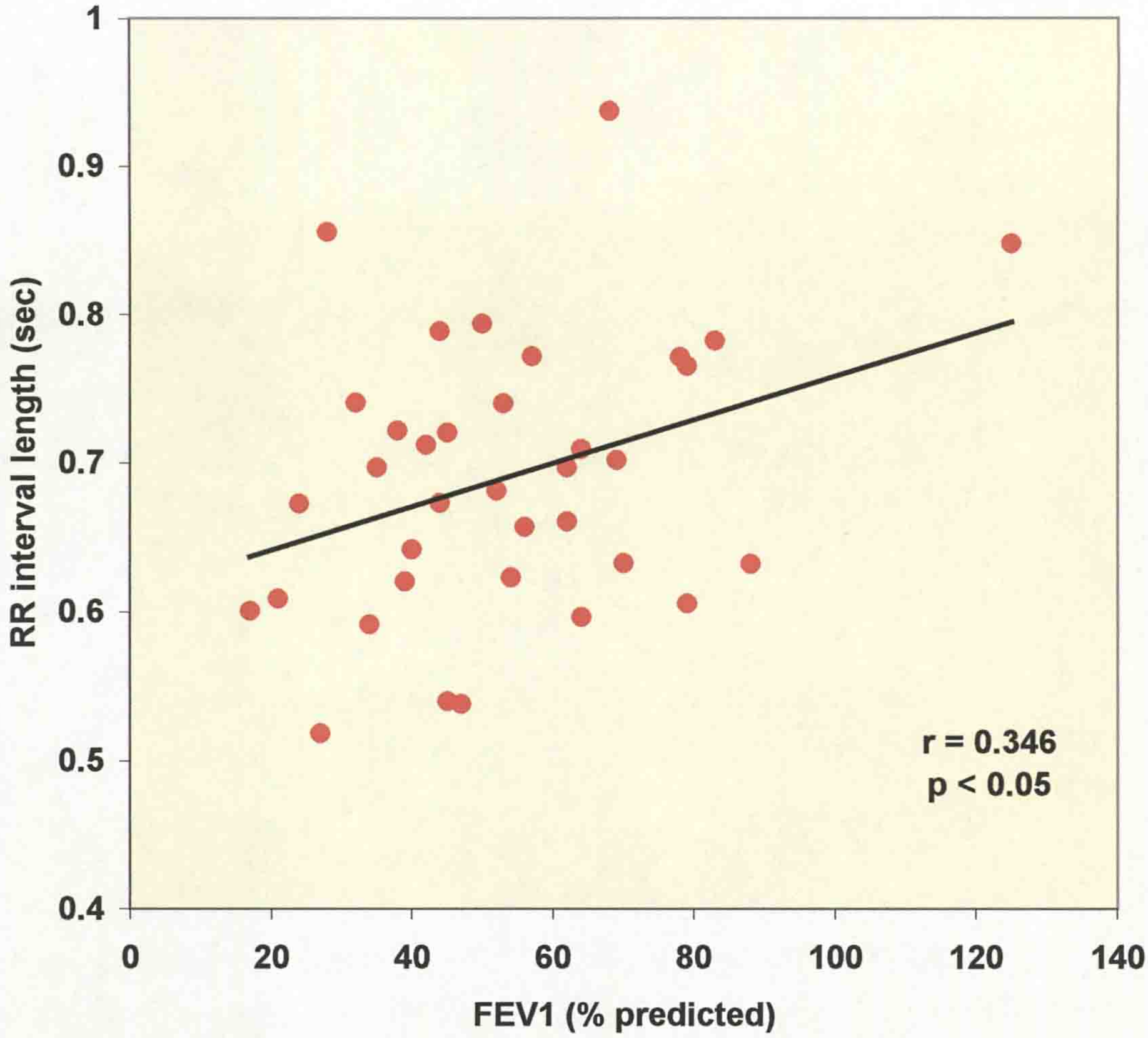
Fasting glucose levels were available for only 48 patients (original group n=49).

Fig 9: Correlation between sympathovagal balance and long-term glucose control in 47 patients



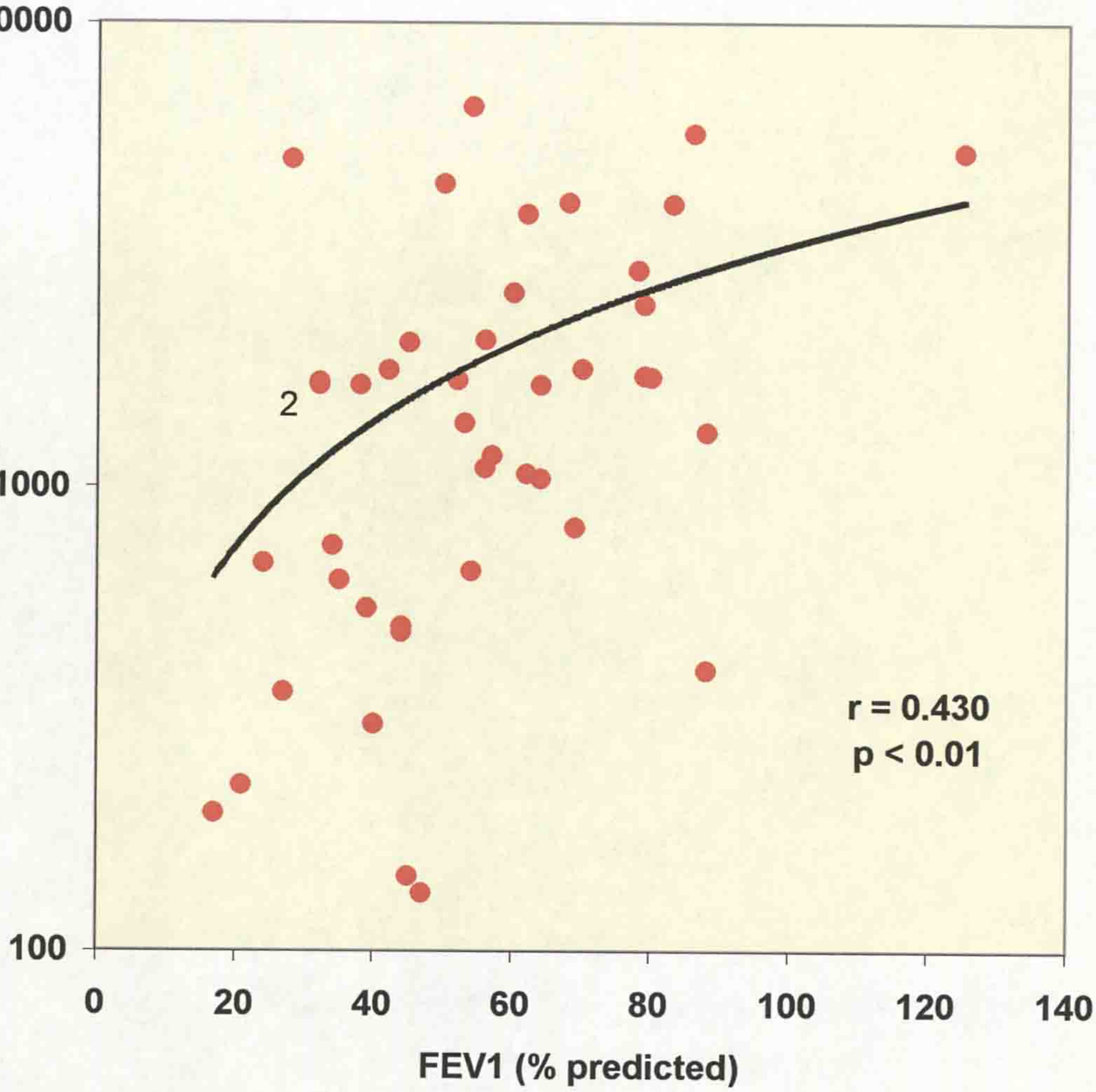
HbA1c levels were available for only 47 patients.

Fig 10: Correlation between heart rate and lung function (FEV1% predicted) in 36 patients



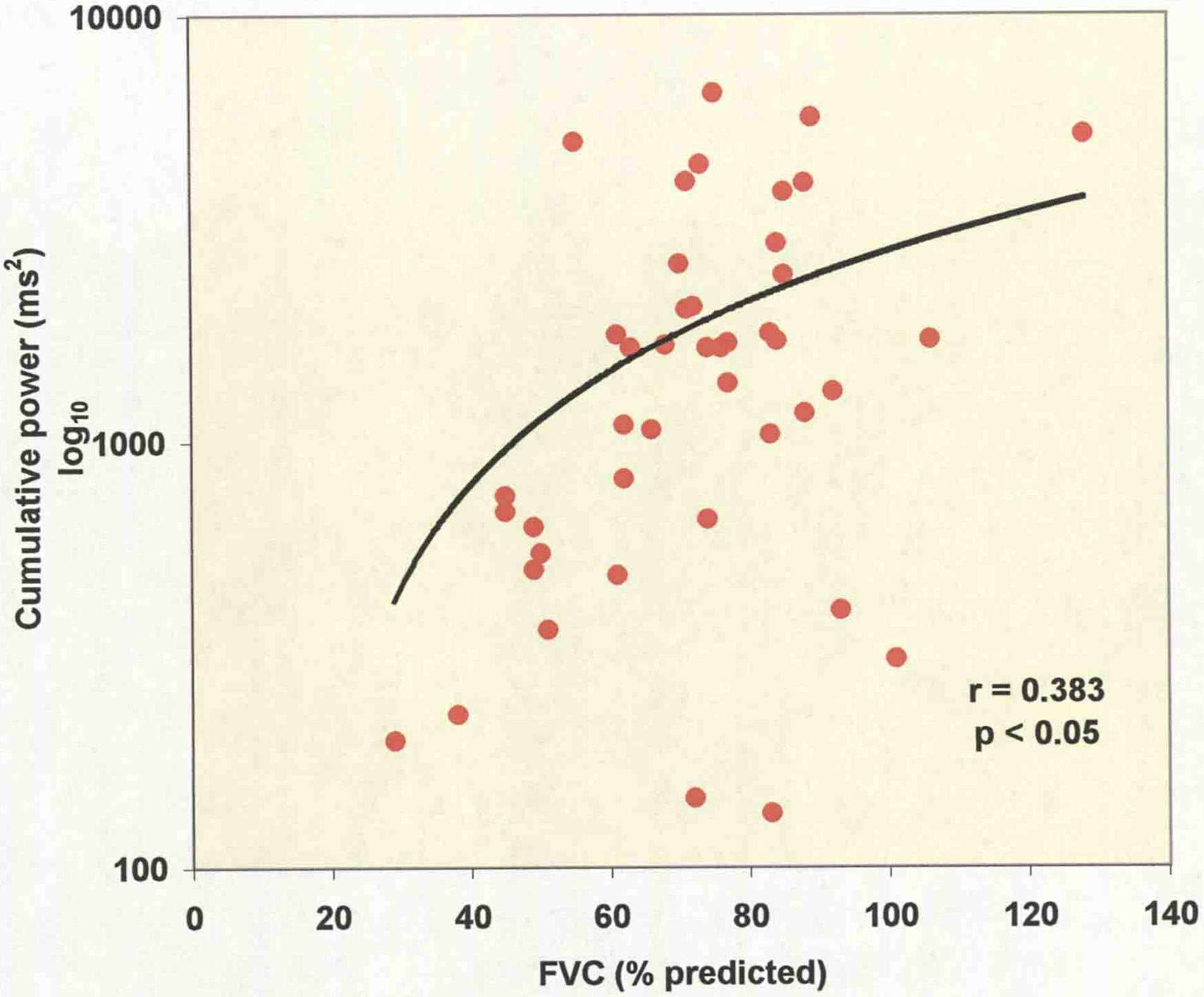
Heart rate and FEV1 (% predicted) were available for only 36 patients as due to computer failure some of the original data was lost. In addition, not all patients were able to perform spirometry.

Fig 11: Correlation between global autonomic tone and lung function (FEV1% predicted) in 43 patients



Cumulative power and FEV1 (% predicted) were available for only 43 patients (original group n=49) due to computer failure with loss of some of the data and because some patients were unable to perform spirometry.

Fig 12: Correlation between global autonomic tone and lung function (FVC% predicted) in 43 patients



Cumulative power and FVC (% predicted) were available for only 43 patients due to computer failure with loss of some of the data and because not all of the patients (original group n=49) were able to perform spirometry.

Comparison between diabetic and non-diabetic patients

Patients were classified as diabetic if they were taking regular insulin. 16 diabetics and 33 non-diabetics performed spectral analysis. There were no significant differences between the 2 groups (Table 14). The number of days spent on intravenous antibiotics over the previous 2 years was the only marker of disease severity that only weakly differentiated the groups (diabetics: mean 160.6 days, range 32-511 versus non-diabetics: 90.2 days, range 7-227, $p<0.052$) (Table 15).

Correlations between spectral parameters and markers of disease severity in diabetic and non-diabetic patients

In the diabetic patients, only the low to high frequency ratio correlated with fasting glucose and HbA1c ($p<0.05$ and $p<0.01$) (Figs 13 and 14); there were no correlations with any other markers of disease severity. By contrast, for non-diabetic patients the remaining spectral parameters correlated with spirometric indices (Figs 15 to 22).

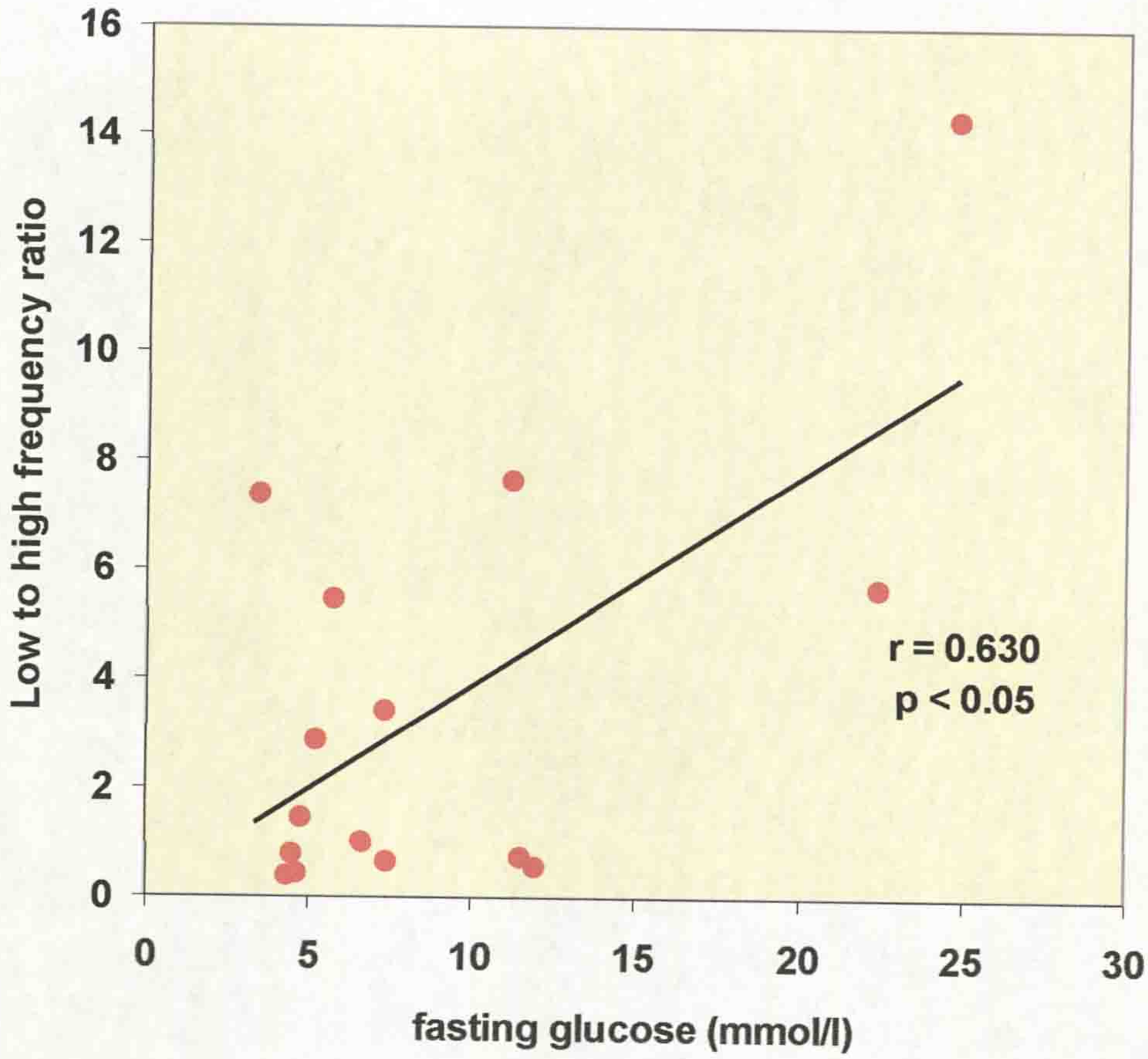
Table 14: Comparison of spectral analysis parameters between diabetics (n=16) and non-diabetics (n=33)

PARAMETER	MEAN VALUE FOR DIABETICS (RANGE)	MEAN VALUE FOR NON-DIABETICS (RANGE)	P VALUE
Total power (ms^2)	508.78 (30.11-2093.23)	831.35 (46.05-4996.54)	0.126
High frequency power (ms^2)	230.70 (1.95-1503.26)	400.96 (7.48-2622.5)	0.204
Low frequency power (ms^2)	278.10 (22.37-860.17)	430.38 (21.83-2374.0)	0.129
Low to high frequency ratio	3.638 (0.393-14.331)	2.580 (0.089-10.751)	0.333
RR interval length (sec)	0.642 (0.509-0.740)	0.699 (0.518-0.937)	0.053
Cumulative power (ms^2)	1456.96 (133.61-5772.1)	2290.38 (145.01-10270)	0.118

Table 15: Comparison of markers of disease severity between diabetics and non-diabetics

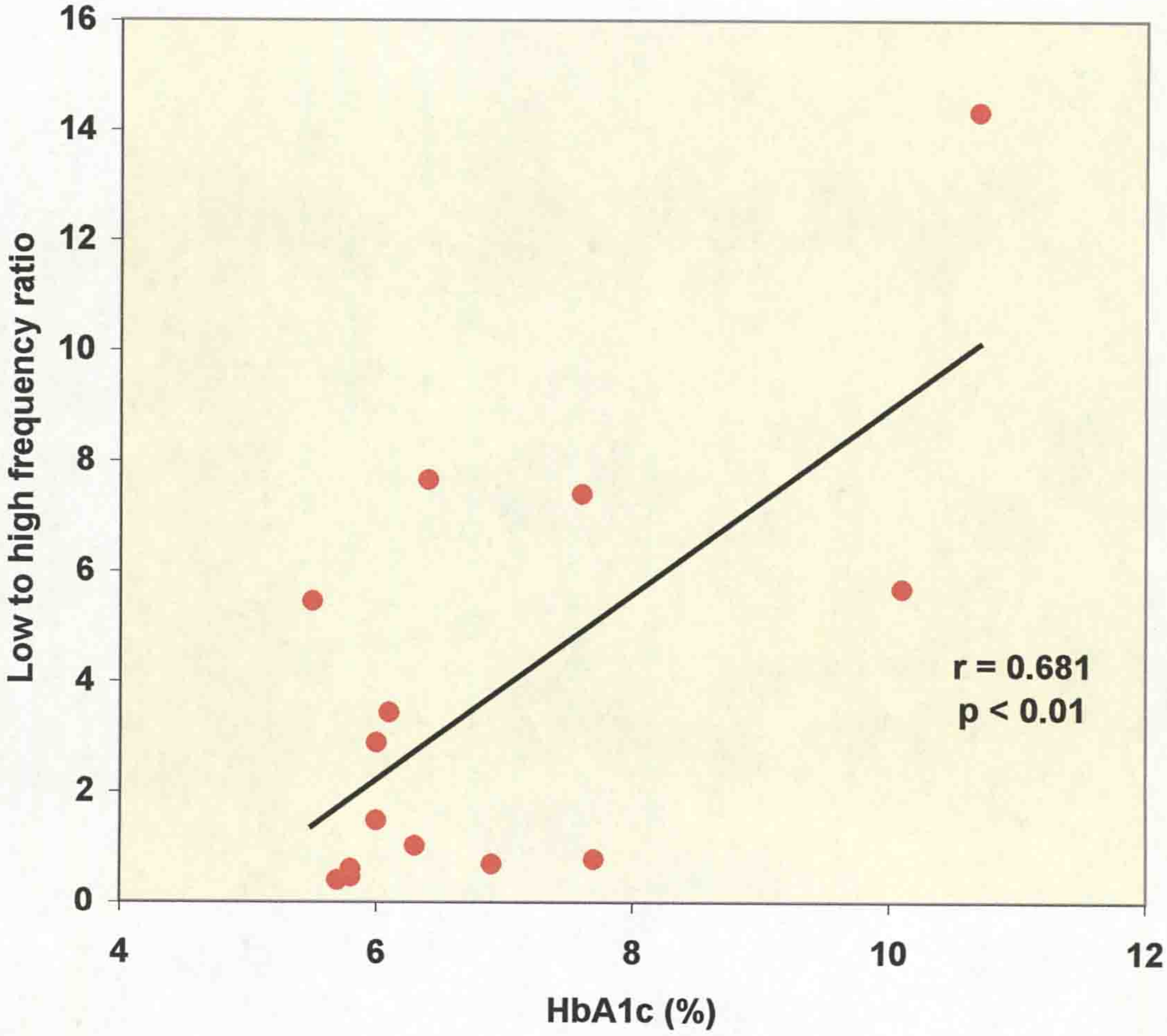
PARAMETER	MEAN VALUE FOR DIABETICS (RANGE)	MEAN VALUE FOR NON-DIABETICS (RANGE)	P VALUE
Age (yrs)	24.5 (19-42)	23.9 (17-41)	0.729
FEV1 (% predicted)	51.4 (19.7-88.0)	56.0 (17-125)	0.559
FVC (% predicted)	76.3 (34.4-106.0)	69.6 (29-128)	0.353
O2 saturations (%)	96.1 (87-100)	96.4 (92-99)	0.767
Body mass index (kg/m^2)	19.2 (13.9-31.2)	21.0 (14.8-31.4)	0.169
Vitamin E (umol/l)	13.3 (1-38)	16.5 (2-37)	0.393
No of courses of iv antibiotics over previous 2 yrs	7.7 (1-17)	6.7 (1-21)	0.489
No of days of iv antibiotics over previous 2 years	160.6 (32-511)	90.2 (7-227)	0.052

Fig 13: Correlation between sympathovagal balance and fasting glucose in 15 diabetic patients



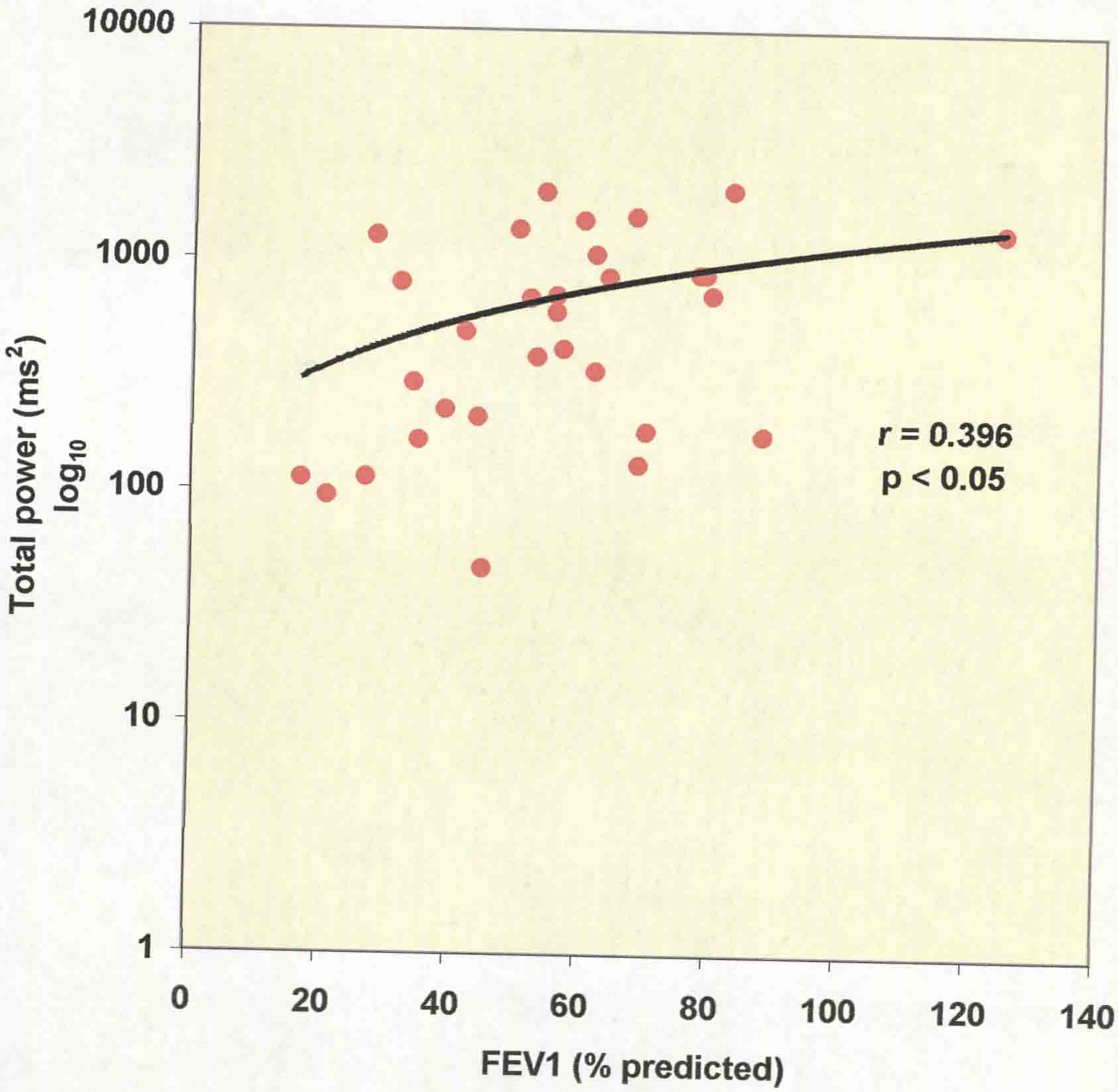
Fasting glucose levels were available for only 15 out of 16 diabetic patients at the time of spectral analysis testing.

Fig 14: Correlation between sympathovagal balance and long-term glucose handling in 14 diabetic patients



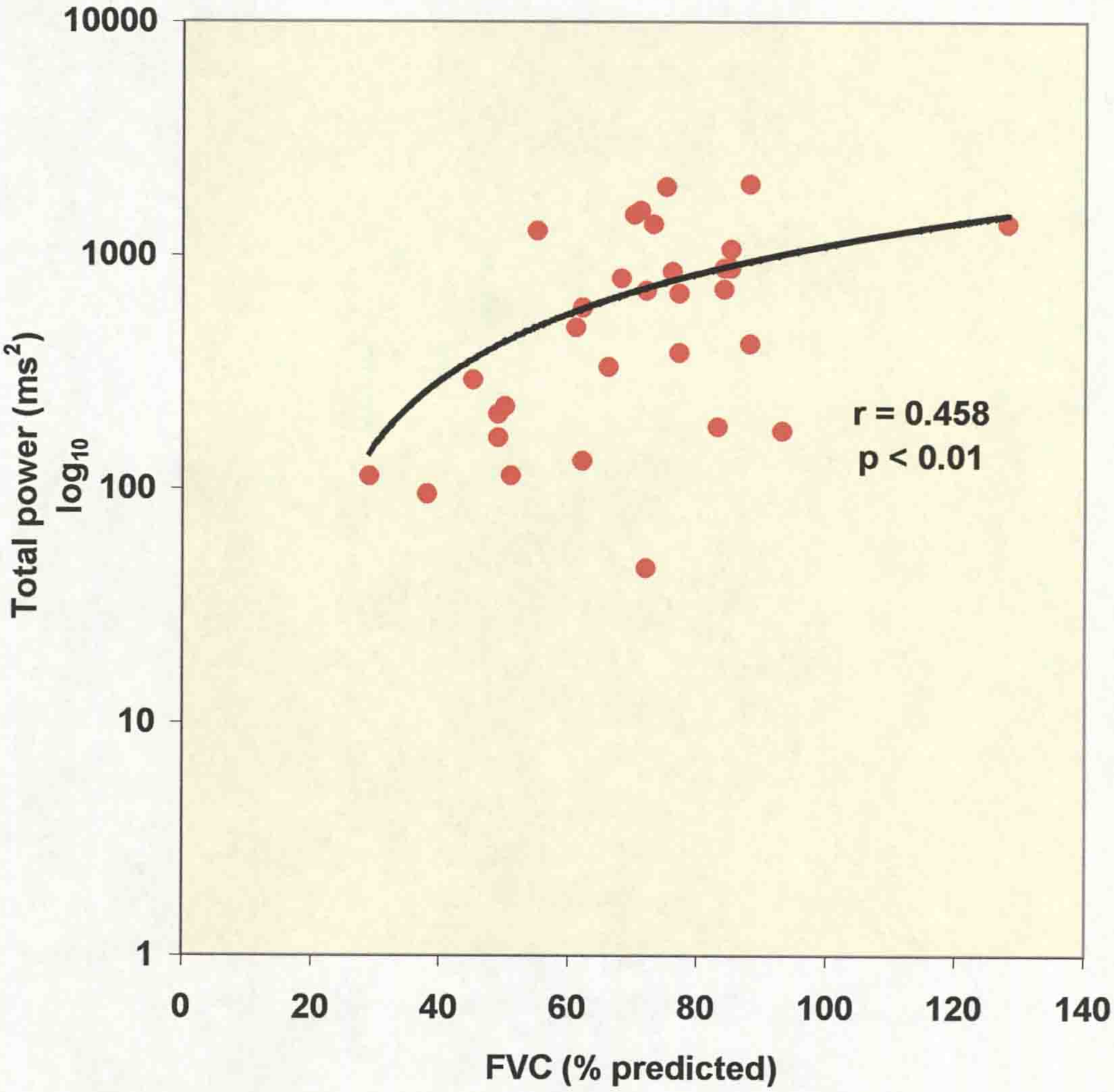
HbA1c levels were available for only 14 out of 16 diabetic patients at the time of spectral analysis testing.

Fig 15: Correlation between total power and lung function (FEV1% predicted) in 31 non-diabetics



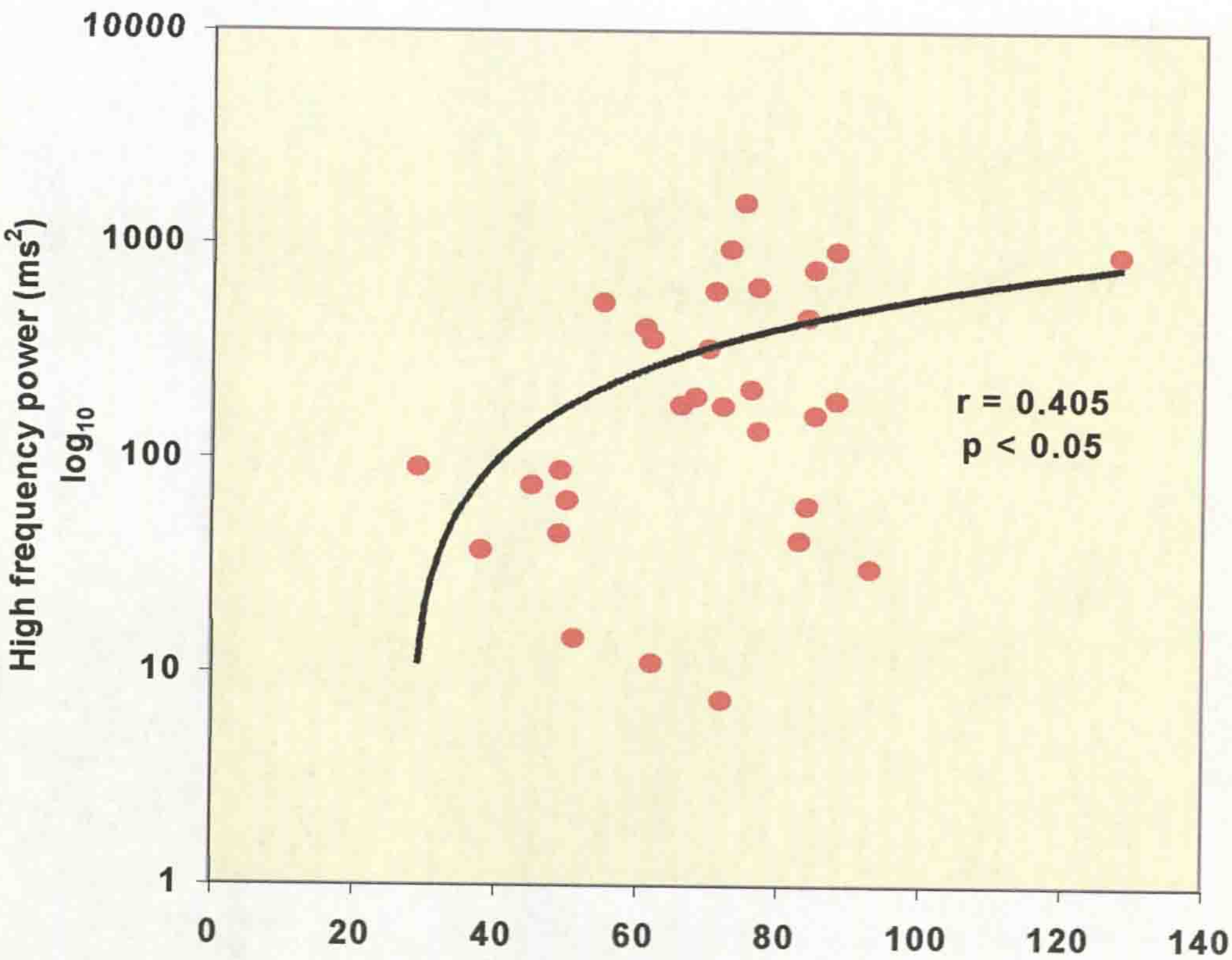
FEV1 (% predicted) was available for only 31 out of 33 non-diabetic patients as not all were able to perform spirometry.

Fig 16: Correlation between total power and lung function (FVC% predicted) in 31 non-diabetics



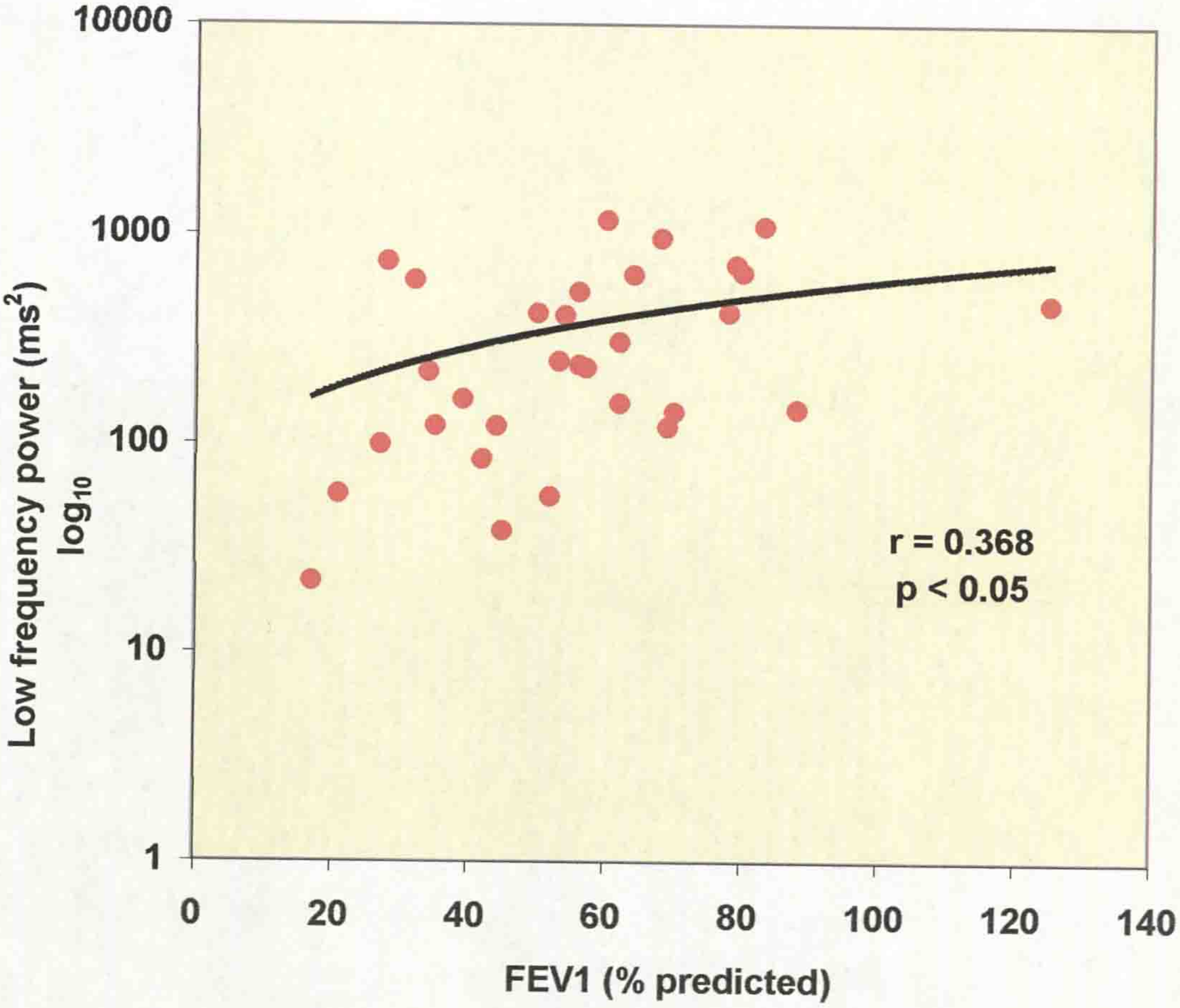
FVC (% predicted) was available for only 31 out of 33 non-diabetic patients as not all were able to perform spirometry.

Fig 17: Correlation between parasympathetic and lung function (FVC% predicted) in 31 non-diabetics



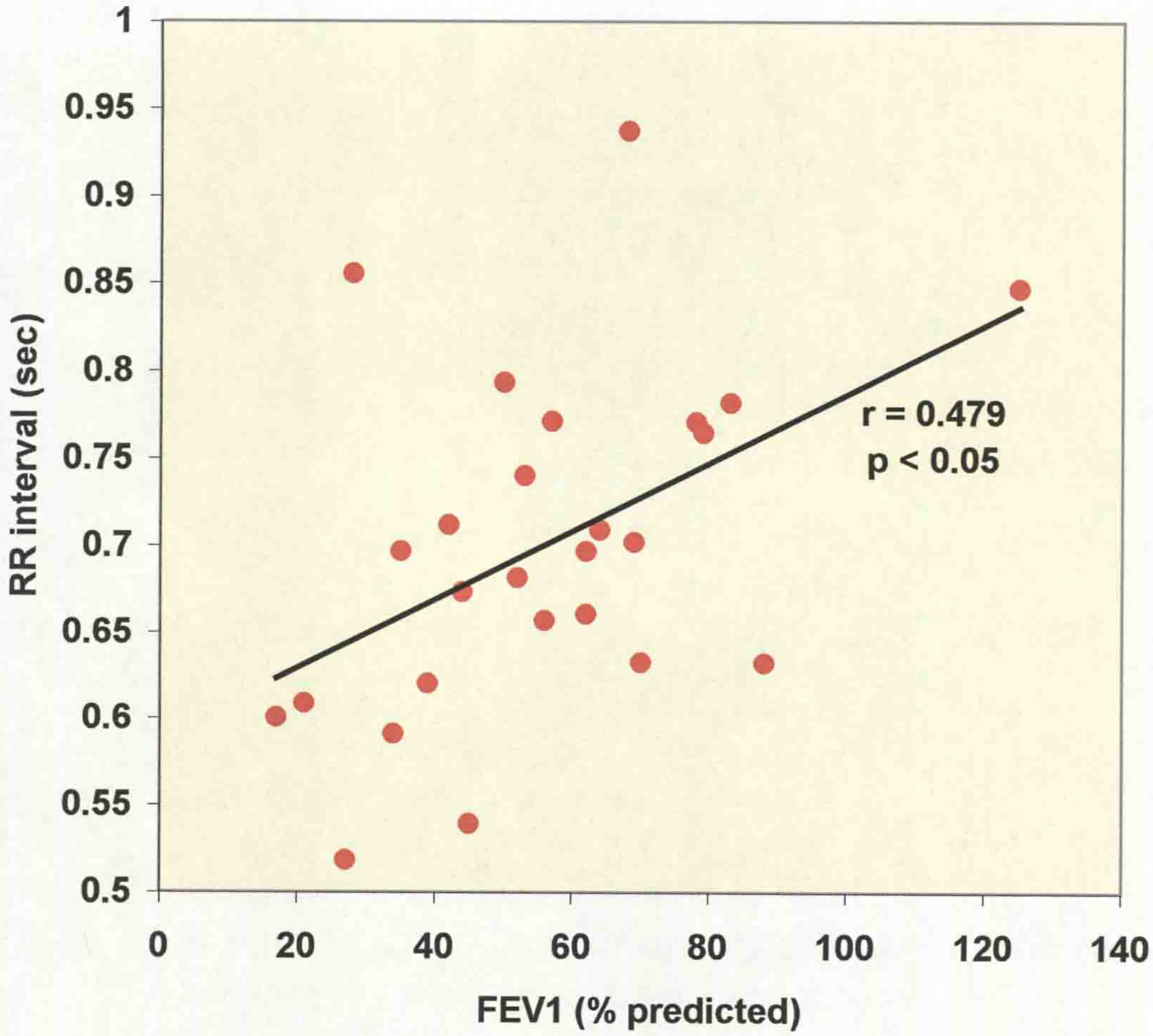
FVC (% predicted) was available for only 31 out of 33 non-diabetic patients as not all were able to perform spirometry.

Fig 18: Correlation between sympathetic function and lung function (FEV1% predicted) in 31 non-diabetics



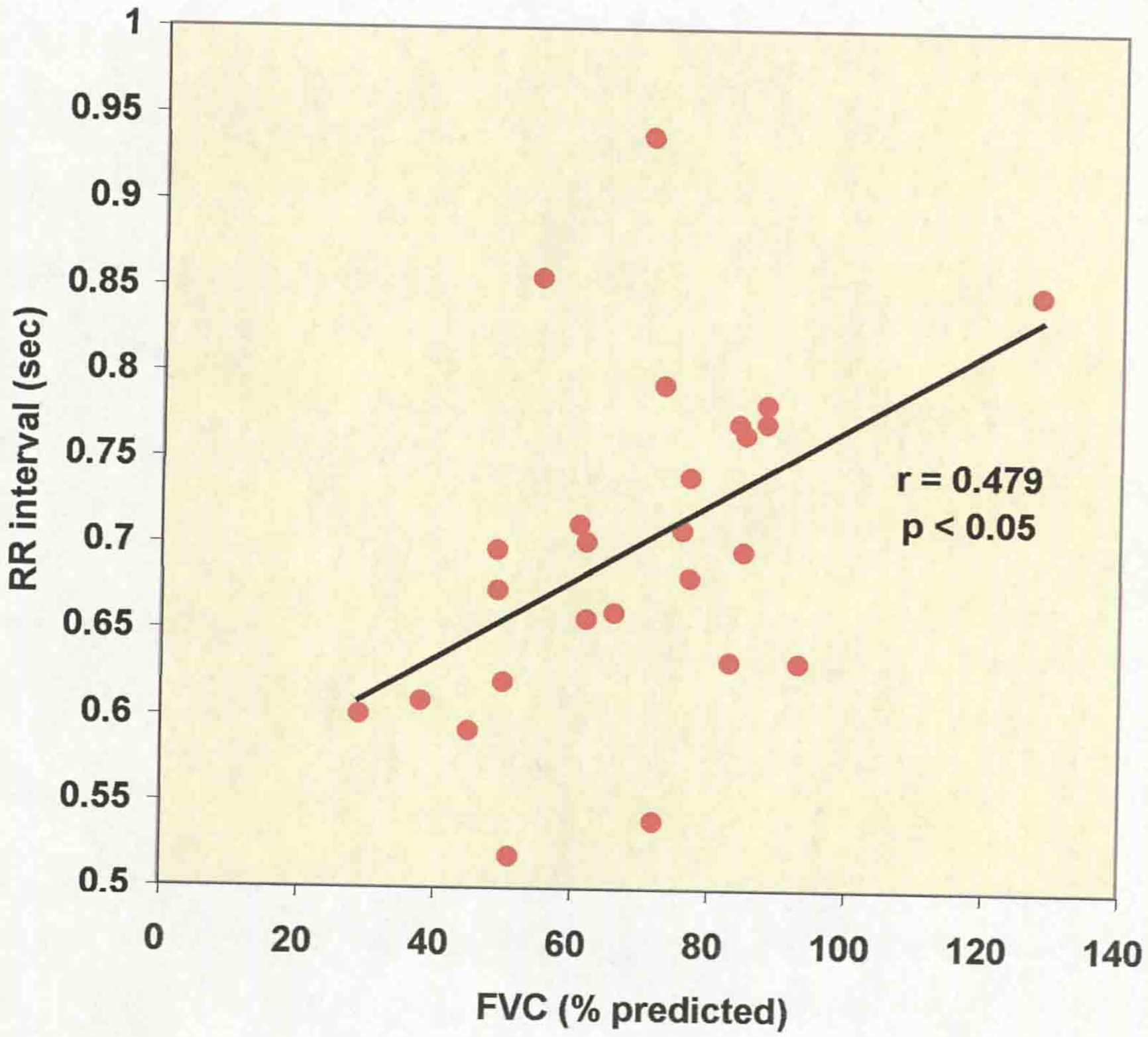
FEV1 (% predicted) was available for only 31 out of 33 patients as not all were able to perform spirometry.

Fig 19: Correlation between heart rate and lung function (FEV1 % predicted) in 26 non-diabetics



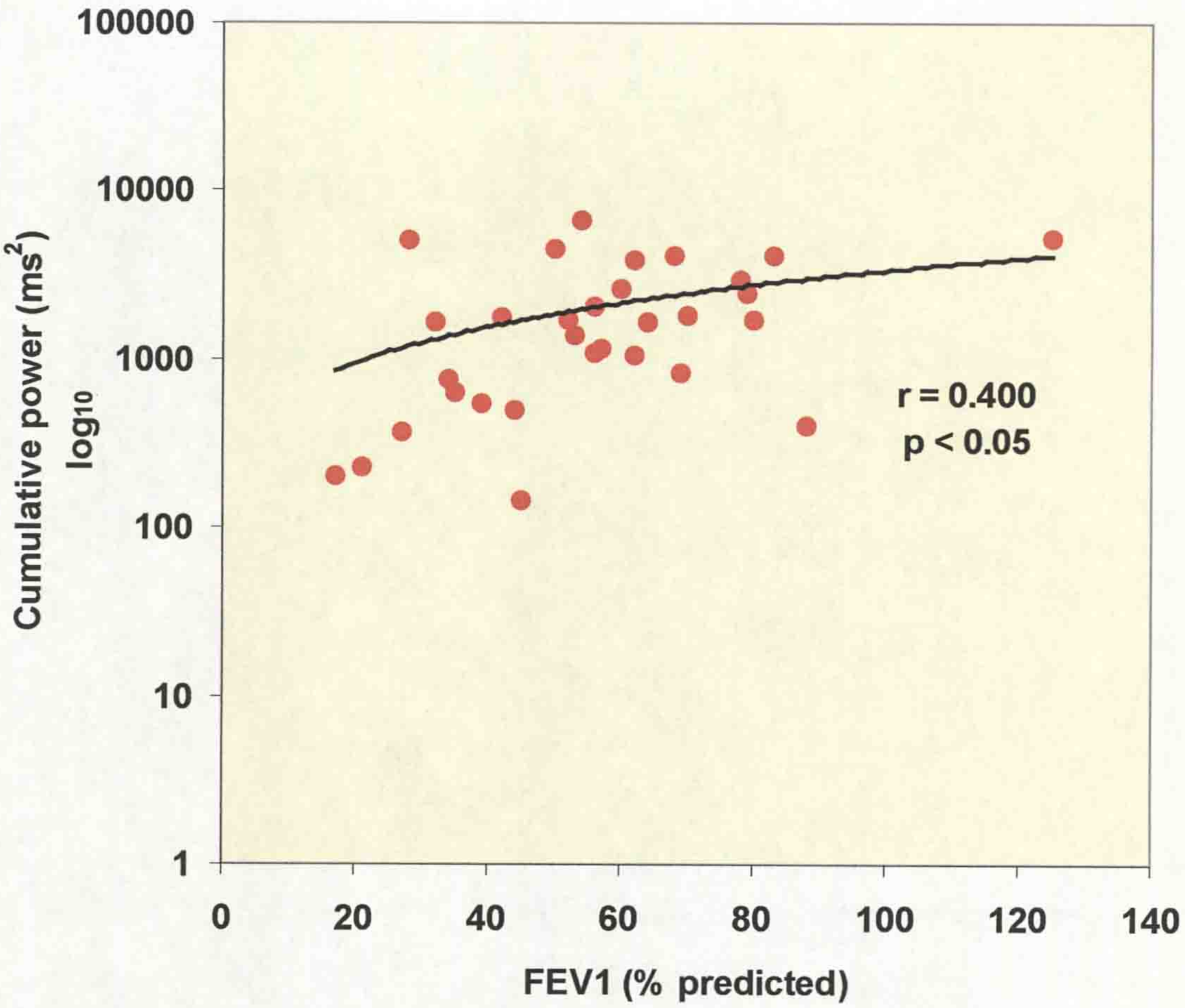
RR interval lengths and FEV1 (% predicted) were available for only 26 non-diabetic patients due to computer failure and because not all patients were able to perform spirometry.

Fig 20: Correlation between heart rate and lung function (FVC% predicted) in 26 non-diabetics



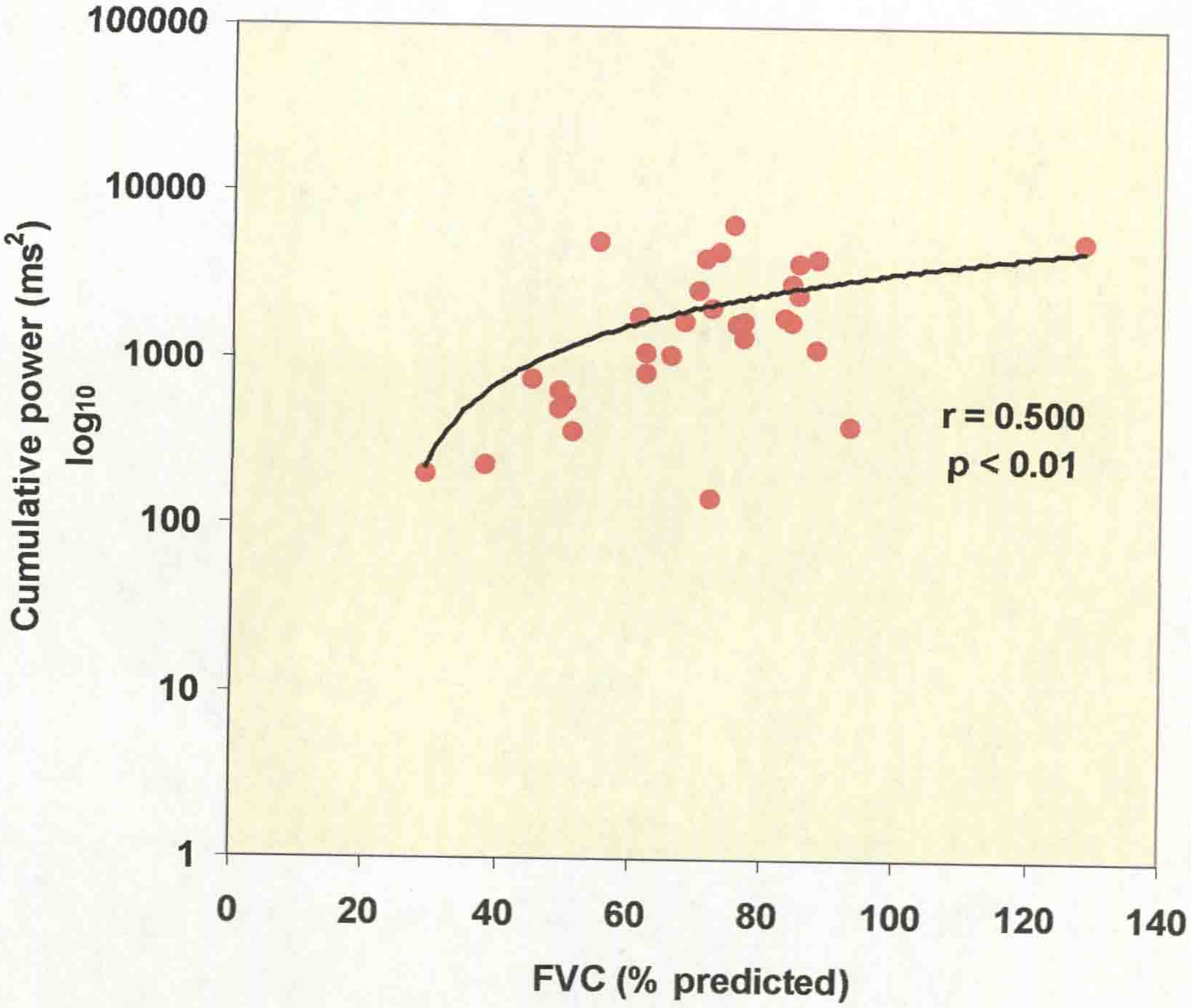
RR interval lengths and FVC (% predicted) were available for only 26 out of 33 non-diabetic patients due to computer failure and because not all patients were available to perform spirometry.

Fig 21: Correlation between global autonomic tone and lung function (FEV1% predicted) in 31 non-diabetics



Cumulative power and FEV1 (% predicted) were available for only 31 out of 33 non-diabetic patients due to computer failure and because not all patients were able to perform spirometry.

Fig 22: Correlation between global autonomic tone and lung function (FVC% predicted) in 31 non-diabetics



Cumulative power and FVC (% predicted) were available for only 31 out of 33 non-diabetics due to computer failure and because not all patients were able to perform spirometry

Summary of results

- All the spectral analysis parameters demonstrated good repeatability making them more reliable than Ewing's tests.

- In both the control and patient groups, the autonomic nervous system demonstrated rapid recovery back to its initial resting state.

- When compared to the control group, the CF patients had significantly worse spectral analysis parameters (with the exception of low frequency power). This cannot be explained by the effects of β-agonists.

- When compared to the *Pseudomonas* colonised patients, those colonised with *B cepacia* had worse autonomic function which was not accounted for by differences in markers of disease severity.

- There were significant correlations between spectral analysis parameters and pulmonary function in the patient group as a whole.

- In the subgroup of diabetic patients, only the LF:HF correlated with glucose handling. By contrast, for the non-diabetics, the remaining spectral parameters all correlated with pulmonary function.

4:2 SPECTRAL ANALYSIS: DISCUSSION

Spectral analysis reduces a signal to the sum of its component sine waves of different amplitudes and frequencies. The power spectrum displays the squared amplitude of these sine waves as a function of frequency. Heart rate fluctuations which reflect modulation of sinus node activity by autonomic mechanisms can be quantified and displayed with this technique (Freeman et al, 1991). Spectral analysis therefore provides a single, specific, non-invasive test of autonomic function measuring both parasympathetic (high frequency power, >0.15Hz) and sympathetic (low frequency power,<0.15Hz) divisions of the autonomic nervous system (Ziegler et al, 1992).

Spectral analysis has already been used to evaluate autonomic function in several chronic diseases. Quantitative evaluation of autonomic neuropathy was performed in 23 diabetics by Lishner et al (1987). A marked reduction in power across all frequencies was observed, the lowest activity being in diabetics with concomitant peripheral neuropathy. A study of children with diabetes has also indicated a reduction of 15% in heart rate variability using power spectral analysis (Karavanaki-Karanassiou, 2001).

Coelho et al (2001) examined autonomic function in 22 cirrhotic patients using 24 hour heart rate variability. Spectral analysis revealed a marked decrease in total, high and low frequency power which was unrelated to aetiology of liver disease but did correlate with severity of disease (Child-Pugh Class A, B and C).

Kurata et al (2000) reported reduced 24 hour heart rate variability across all frequencies in 44 patients with chronic renal impairment when compared to controls.

In patients with chronic congestive cardiac failure, Saul et al (1988) showed that heart rate spectral power was markedly reduced at all frequencies examined and virtually absent at frequencies greater than 0.04Hz.

Recent work in adult CF patients by Tattersall et al (2001) has demonstrated a strong positive correlation between all parameters of spectral power and pulmonary function ($p<0.001$).

Controls

I have included a control group in my study as there are no published normal range values for spectral analysis parameters (Schumer et al, 1998). An age-related decline has been observed in controls in the power of heart rate fluctuations across all frequencies by Lishner et al (1987). However, the ages of these subjects ranged from 41 to 78 years. The age range of my controls was 22 to 39 years, thus accounting for the lack of correlation with age in my study.
My data has also shown a wide range in spectral parameters in the control group reflecting marked inter-individual variability.

Unlike Ewing's tests, little data exists on the repeatability of spectral parameters. Ziegler et al (1992) argued that the results of all indices of spectral analysis described a log normal distribution and hence the standard deviation factor (SDF_{intra}) was taken as a measure of repeatability using the formula:

SDFintra = antilog of the square root of the mean of the variances resulting from each triad of log-transformed values

My values of 1.54 and 1.51 for high and low frequency power compares favourably with 1.58 and 1.62 from Ziegler's study. No data was given for total autonomic or cumulative power but again my values of 1.39 and 1.27 demonstrate good repeatability.
I found that the coefficient of variation of 6.33% for the RR interval in my study was similar to that of 5% for the average heart rate over a 24 hour period in a study of 22 normal controls by Huikuri et al (1990).

Spectral parameters were compared in the initial and final supine positions of the 15 minute orthostatic load. No significant differences were found, indicating the autonomic nervous system's ability to rapidly return to its baseline level.

Effect of bronchodilators

Many CF patients take nebulised bronchodilators (eg salbutamol) as part of their regular daily therapy. Jartti et al (1997*a*) observed that 600mcg of inhaled salbutamol increased sympathetic dominance in the cardiovascular systems of 8 children with bronchial asthma. More recently, Eryonucu et al (2001) studied the effects of 200mcg inhaled salbutamol and 500mcg inhaled terbutaline on 20 adult asthmatics. Spectral analysis was performed for each 5 minute segment, 5 minutes before inhalation and 5, 10, 15, 20, 25 and 30 minutes afterwards. The low frequency (sympathetic component) and low to high frequency ratio increased at 5, 10, 15 and 20 minutes after inhalation of salbutamol and terbutaline. Thereafter, the effects decreased. Similarly in my study, the administration of nebulised salbutamol to a control subject resulted in an increase in low frequency power . This returned to baseline levels at 20 minutes. Heart rate remained elevated at 15 to 20 minutes but had significantly decreased at 60 minutes and the effects had certainly worn off by 3 hours. Hence spectral analysis was performed on CF patients at least 3 hours after administration of nebulised ß-agonists.

Dagnone and Parlow (1997) showed that inhalation of 80mcg of ipratropium bromide through a volumatic device did not alter the autonomic control of the cardiovascular system in healthy male subjects.

Patient group

As with controls, patients also demonstrated a wide inter-individual variability in spectral parameters. As there is no established set of normal values, the lower limit of normal for each parameter was taken as the 5^{th} centile calculated from the control group, as used by Lanting et al (1990*b*) in a study of 62 controls and by May and Arildsen (2000) using 194 controls. Using this as my cut-off point, overall a higher percentage of patients had abnormalities than were discovered with Ewing's tests, the highest percentage being for high frequency power. This is in agreement with the findings of several other studies. Lishner et al (1987) compared the results of spectral analysis parameters with results from a one deep

breath test in 23 diabetics. The reduction in fluctuations in these patients as compared with controls was greatest in the high frequency range by a factor of 6. For lower frequencies, the reduction was somewhat less pronounced. Furthermore, 6 patients with normal results on the deep breath test had depressed autonomic function as diagnosed by spectral analysis. By contrast, there was only 1 patient with an abnormal deep breath test and normal spectral parameters. A prospective study by Karamitsos et al (1998) investigated the natural history of spectral indices and cardiovascular reflex tests in diabetics with recently diagnosed asymptomatic autonomic neuropathy over a period of 2 years at 3 month intervals. Most spectral indices deteriorated significantly in comparison to baseline at month 12, whilst the values of cardiovascular reflex tests displayed deterioration at a later stage, months 15 to 21. Ziegler et al (2001) estimated the accuracy of a battery of blood pressure and heart rate variability indices (spectral measures) obtained in different subgroups of diabetics classified according to Ewing's tests. Subjects with borderline or definite cardiovascular autonomic neuropathy showed similar degrees of alterations of both blood pressure and heart rate variability. Subjects with normal Ewing's tests results also showed an altered relationship between blood pressure and heart rate, representing an early stage of cardiovascular autonomic neuropathy undetected by conventional tests. A more recent study by Takase et al (2002) of 18 diabetics reported that high frequency spectra were markedly decreased even in patients classified as having early vagal damage on the basis of Ewing's battery.

Vita et al (1999) examined 30 chronic uraemic patients on periodic bicarbonate haemodialysis using Ewing's battery and power spectral analysis. A significant reduction in low frequency power was evident in patients without Ewing's test-proven autonomic neuropathy, suggesting early sympathetic involvement that traditional tests were unable to detect.

As for the control group, recovery of the autonomic nervous system back to its baseline level was also apparent in the patient group.

Comparison with controls

Patients had significantly worse values for all spectral analysis parameters with the exception of low frequency power (sympathetic activity). This could theoretically be due to the effects of patients taking regular bronchodilators; Jartti et al (1997*b*) observed that 200mcg of inhaled salbutamol three times a day for 2 weeks not only increased sympathetic drive in asthmatic children but the effect was also evident 18 hours after cessation of treatment, reflecting the adaptation of organ responses to regular therapy or altered central autonomic regulation. However, my data showed that a comparison between patients who were and who were not taking regular bronchodilators revealed no statistically significant differences in spectral parameters. It may be explained by the fact that either parasympathetic dysfunction precedes sympathetic dysfunction or that low frequency power is not a good discriminator between patients and controls (only 12.2% of patients had abnormal low frequency power). This has been susbstantiated by some previous investigators. For example, Lishner et al (1987) in their study of 23 diabetics and 22 controls reported that the reduction in fluctuations in the low frequency range was less pronounced than for higher frequencies. Saul et al (1988) reported that in patients with chronic congestive cardiac failure, although heart rate spectral power was markedly reduced at all frequencies compared to controls, heart rate fluctuations at very low frequencies less effectively discriminated heart failure patients from controls, indicating there was diminished vagal but relatively preserved sympathetic modulation of heart rate.

There is also some controversy as to what exactly the low frequency component represents. Experiments in man and conscious dogs indicate that low frequency power can be used as an index of sympathetic activity (Pagani et al, 1986). However, using pharmacological means, Pomeranz et al (1985) in their study of 8 male control subjects demonstrated that low frequency power may be a combination of sympathetic and parasympathetic activity. In the supine position, atropine reduced the low frequency area by 84%; addition of propranolol after this did not produce any further reductions in low frequency power. The administration of propranolol first did not result in any significant drop in low

frequency power in the supine position. Addition of atropine then reduced this. Thus in the supine position, low frequency heart rate fluctuations are largely mediated by parasympathetic activity. By contrast, in the standing posture, the suppression of the low frequency peak by atropine was only –72% of baseline, and this was further diminished by propranolol to –89% of baseline. Propranolol alone reduced this by 73%, indicating the presence of a strong sympathetic influence on low frequency fluctuations in the standing position.

Burkholderia cepacia versus *Pseudomonas* colonised patients

Unlike Ewing's tests, spectral analysis was able to reveal significantly worse autonomic function in the *B cepacia* colonised patients for total power, high and low frequency power and cumulative power. This was not due to any significant differences in markers of disease severity between the 2 patient groups. Therefore, this would firstly substantiate the work of previous studies showing the increased sensitivity of spectral analysis, and secondly suggest that microbiological status may directly impact on the severity of autonomic dysfunction in CF.

Male versus female patients

A previous study by Ryan et al (1994) demonstrated that high frequency power was higher in healthy women compared to males. Jensen-Urstad et al (1997) revealed that low frequency power was lower in female healthy subjects compared to males. These findings were also reflected in my patient group, but the differences were not statistically significant.

Correlations between spectral parameters and markers of disease severity

Significant correlations existed between spirometric indices and total autonomic function, high and low frequency power, RR interval length and cumulative power in the patient group as a whole. This has also recently been shown in a pilot study of 26 adult CF patients (Tattersall et al, 2001) in which there were strong correlations between total autonomic function and FEV1% predicted ($r=0.62$, $p<0.01$) and FVC% predicted ($r=0.68$, $p<0.01$), and these also correlated with sympathetic and parasympathetic activity. Although there was a relationship between low to high frequency ratio and fasting glucose levels and long-term glucose handling in all patients ($r=0.409$, $p<0.01$ and $r=0.476$, $p<0.001$) and in diabetics ($r=0.63$, $p<0.05$ and $r=0.681$, $p<0.01$), there were no correlations between spectral indices and HbA1c. This may be because CFRDM is an entity distinct from conventional diabetes mellitus (see earlier). However, several previous studies have also failed to show a relationship between HbA1c or duration of diabetes. For example, Lishner et al (1987) found no difference in power spectral analysis parameters between patients with hyperglycaemia for more than 10 years duration and those with hyperglycaemia of less than 10 years. Similarly, Pagani et al (1988) found no relationship between spectral components and duration of disease and severity as reflected by HbA1c in 49 diabetics. In another study of 28 diabetic patients, there was no influence of HbA1c and duration of diabetes on baroreceptor frequency (0.04 to 0.12 Hz) (Lanting et al, 1990*b*).

Although the mean value for vitamin E levels in this study (13.1umol/l) was lower than the normal range (14 to 47umol/l), we found no relationship with spectral parameters. This is in contrast to other studies which have demonstrated the effects of vitamin E deficiency on neurological function in CF patients. Sitrin et al (1987) reported abnormal eye movements, diminished reflexes, decreased vibratory and position sense, ataxia and muscle weakness in adult CF patients; treatment with vitamin E partially corrected these deficits. Cynamon et al (1988) evaluated neurological function in 18 CF patients. Sural nerve conduction

latency was increased and nerve action potential decreased in the vitamin E deficient group. However, the effects of vitamin E deficiency on autonomic function in CF have not previously been investigated.

Recent studies have indicated abnormalities in autonomic function both in anorexic and obese patients. Casu et al (2002) evaluated changes in autonomic control of heart rate after postural variation by means of spectral analysis of RR interval variability in 13 female anorexic patients (mean BMI 16.9±2.6). Compared with controls the high frequency component in patients did not change significantly on changing posture. By contrast, Surrenti et al (2002) reported that in obese subjects (mean BMI 45.72±7.48), spectral analysis of RR interval variability showed an increase in high frequency components in the standing and lying positions compared to controls. I failed to find any correlations between spectral parameters and BMI in my patients. However, the mean BMI in my study was 20.4 (±4), significantly different from the above studies.

Finally, as for the patient group as a whole, with the exception of low to high frequency ratio, there were significant correlations between other spectral parameters and lung function in non-diabetics, indicating that at least in those patients with normal glucose handling, autonomic imbalance is not an important factor contributing towards the disease process.

CHAPTER 5: OPHTHALMIC SYSTEM

5:1 OPHTHALMIC SYSTEM: RESULTS

5:2 OPHTHALMIC SYSTEM: DISCUSSION

5:1 OPHTHALMIC SYSTEM: RESULTS

Control subjects

14 control subjects took part in the study, 4 male and 10 female. Their mean age was 29.8 years (range 22 to 38 years). The mean pupil diameter percent (PD%) was 59.69% (range 50 to 67.39%). There was no correlation with age (r=-0.346, p=NS).

Reproducibility study

The eyes of 12 subjects were photographed twice at 15 minute intervals. Table 1 shows their PD% values. The mean coefficient of variation of PD% was 2.6%.

Repeatability study

The eyes of 11 subjects were photographed twice one week apart. Table 2 shows their PD% values. The mean coefficient of variation of PD% was 3.9%.

Effect of bronchodilators on pupil size

Sympathetic activity causes pupil dilatation whilst parasympathetic activity results in pupil constriction. Therefore, nebulised salbutamol (a β-agonist) and ipratropium bromide (an anticholinergic agent) via a facemask could potentially affect pupil size in CF patients taking these drugs. To investigate this further, nebulised salbutamol (5mg) and ipratropium bromide (0.5mg) were administered through a facemask to a control subject and PD% calculated at intervals over a 30 minute period. Figs 1 and 2 indicate there was little effect on PD% during this time interval. However, pupil photographs in CF patients were taken at least 30 minutes post nebuliser therapy.

Table 1: Reproducibility study in 12 control subjects

SUBJECT	PD% (time=0)	PD% (time=15 mins)	Mean of PD%	Standard deviation	Coefficient of variation (%)
1	50.0	50.0	50	0	0
2	66.67	66.67	66.67	0	0
3	62.5	64.0	63.25	1.06	1.68
4	65.22	69.56	67.39	3.07	4.56
5	58.33	58.33	58.33	0	0
6	69.23	64.0	66.62	3.7	5.55
7	54.55	54.55	54.55	0	0
8	60.87	66.67	63.77	4.1	6.43
9	58.33	58.33	58.33	0	0
10	52.17	56.52	54.35	3.08	5.67
11	58.33	60.0	59.17	1.18	1.99
12	54.55	59.1	56.82	3.22	5.67

Mean coefficient of variation = 2.6%

Table 2: Repeatability study in 11 control subjects

SUBJECT	PD% (time=0)	PD% (time=1 week)	Mean of PD%	Standard deviation	Coefficient of variation (%)
1	50.0	54.54	52.27	3.21	6.14
2	63.25	64.0	63.62	0.53	0.83
3	67.39	68.87	68.13	1.05	1.54
4	58.33	58.33	58.33	0	0
5	62.5	50.0	56.25	8.84	15.7
6	66.62	74.1	70.36	5.29	7.52
7	54.55	54.35	54.45	0.14	0.26
8	58.33	62.5	60.41	2.95	4.88
9	54.35	54.54	54.45	0.13	0.24
10	53.85	50.0	51.93	2.72	5.24
11	59.16	58.33	58.75	0.59	1.00

Mean coefficient of variation = 3.9%

Fig 1: Evolution of pupil diameter percent over time after administration of nebulised salbutamol to a control subject

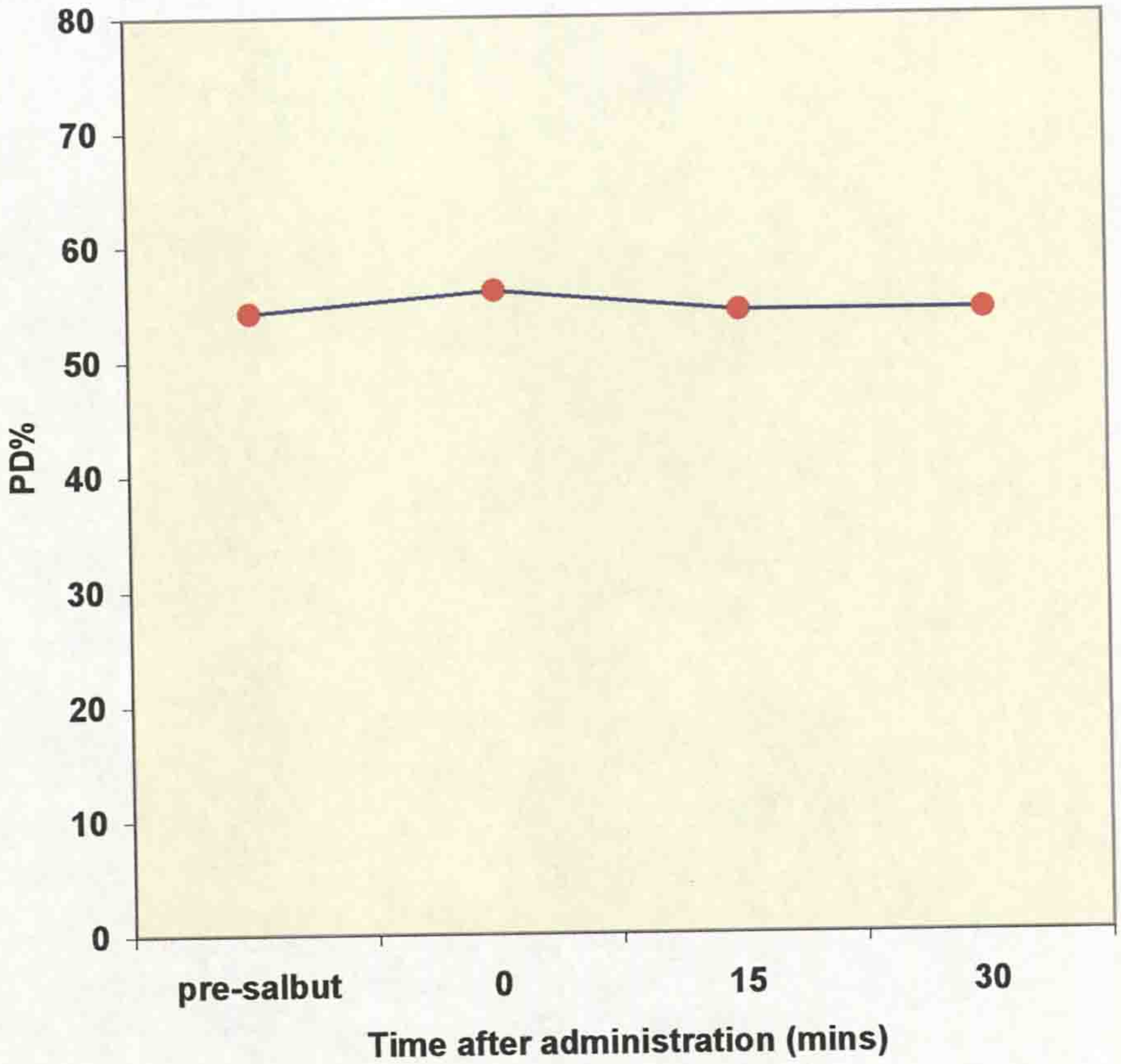
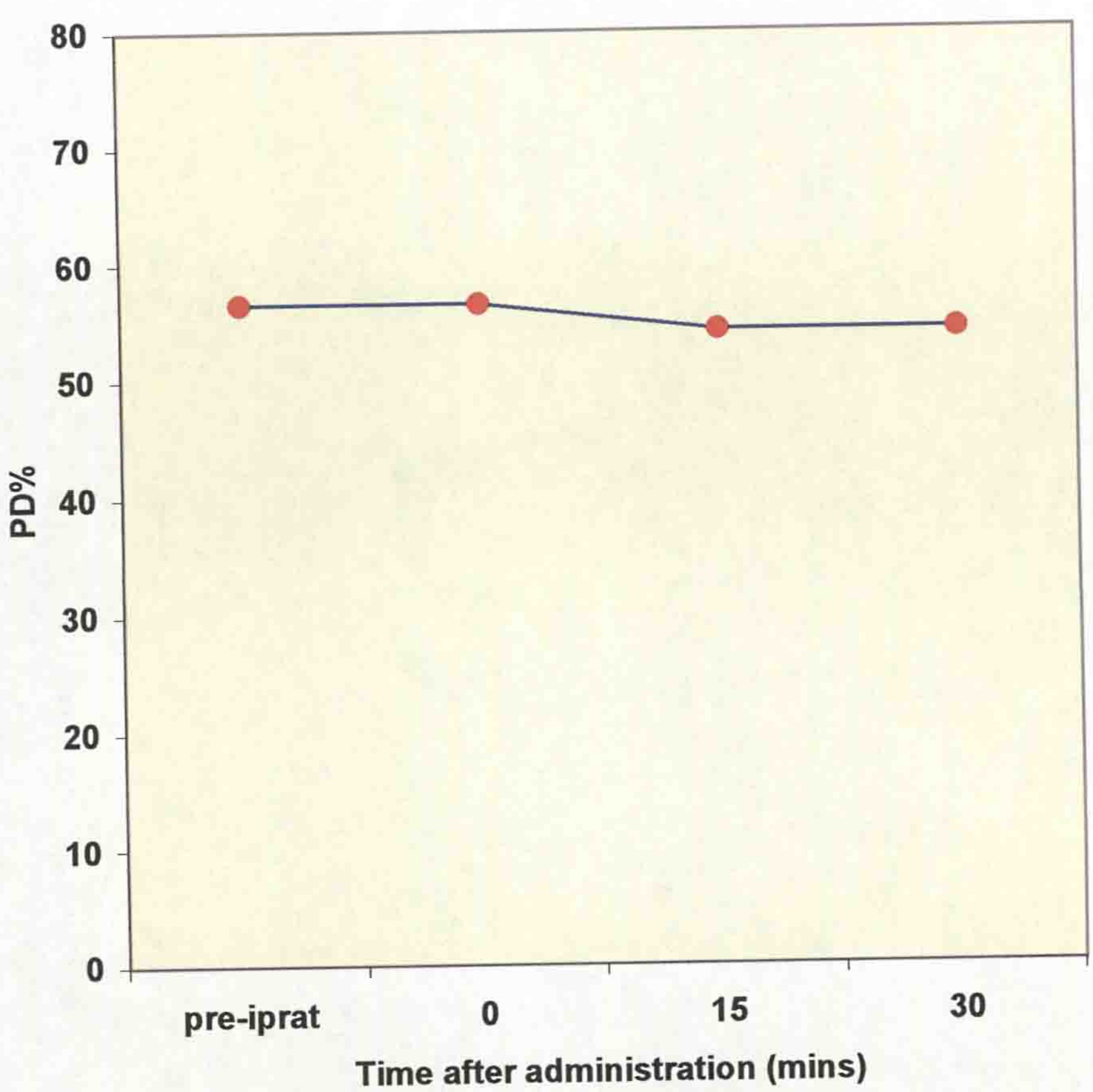


Fig 2: Evolution of pupil diameter percent over time after administration of nebulised ipratropium to a control subject



Patients

35 patients were included, 15 male and 20 female. All patients were in a stable clinical state. The mean age was 25.8 years (range 19 to 51 years). Mean PD% was 63.15% (range 50 to 73.9%) (Fig 3). There were no significant differences in PD% between male and female patients (males mean 64.16%, females 62.38%, p=NS).

Table 3 shows mean values plus ranges for markers of disease severity in the entire patient group.

Comparison with control subjects

There was no significant difference in PD% between the control and CF groups (mean 59.69% versus 63.15%, p=NS) (Fig 4).

Comparison between patients colonised with *Burkholderia cepacia* and *Pseudomonas aeruginosa*

25 patients (mean age 24.4 years, range 19 to 51 years) were colonised with *Pseudomonas aeruginosa*. 10 were colonised with *Burkholderia cepacia*. No significant differences existed between the two groups for PD% (*Pseudomonas* mean 62.79%, *B cepacia* mean 64.03%, p=NS) (Fig 5) or any marker of disease severity (Table 4).

Fig 3: Pupil diameter percent in the patient group (n=35)

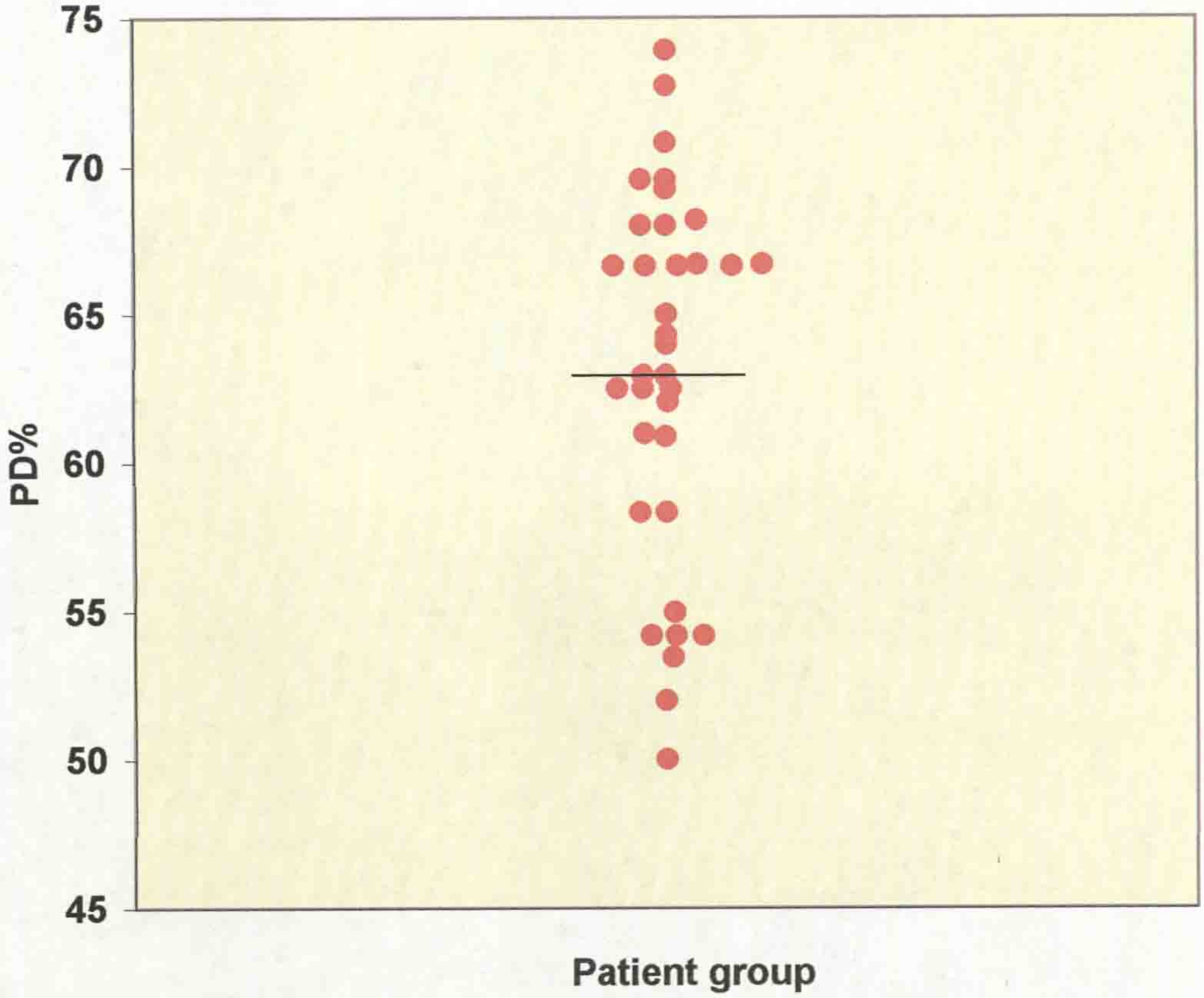


Table 3: Markers of disease severity in the patient group

PARAMETER	MEAN VALUE (RANGE)
FEV1 (% predicted)	53.4 (17-88)
FVC (% predicted)	70.1 (29-106)
O2 saturations (%)	96.7 (92-100)
Body mass index (BMI)	20.3 (13.9-31.2)
Fasting glucose (mmol/l)	7.1 (4-24.8)
HbA1c (%)	6.4 (5.5-10.7)
Vitamin E (umol/l)	16.4 (1-38)
No of courses of intravenous antibiotics over previous 2 yrs	6.4 (1-17)
No of days on iv antibiotics over previous 2 yrs	101.2 (6-351)

Fig 4: Comparison of pupil diameter percent between controls and patients

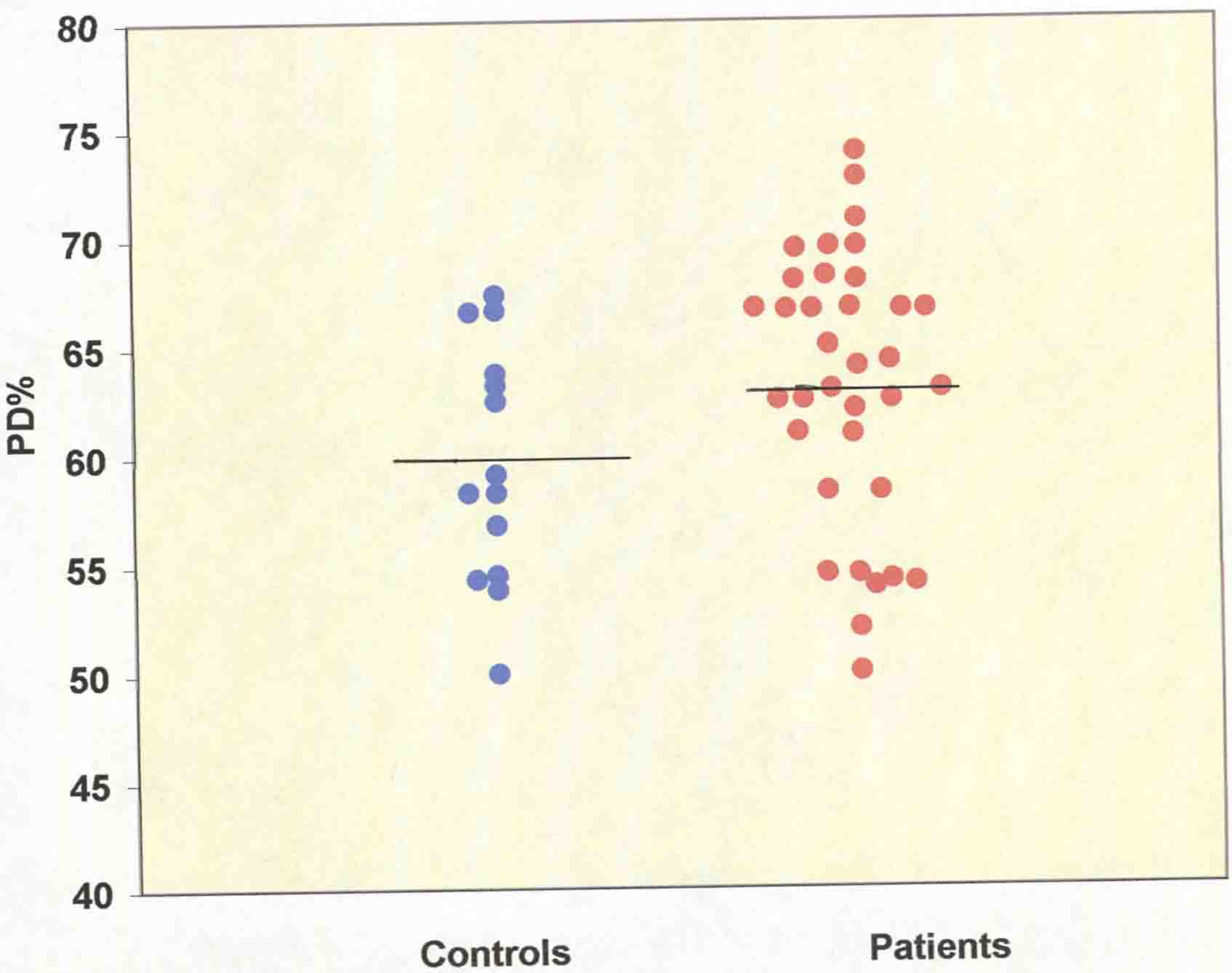


Fig 5: Comparison of pupil diameter percent between *Pseudomonas aeruginosa* (n=25) and *B cepacia* (n=10) colonised patients

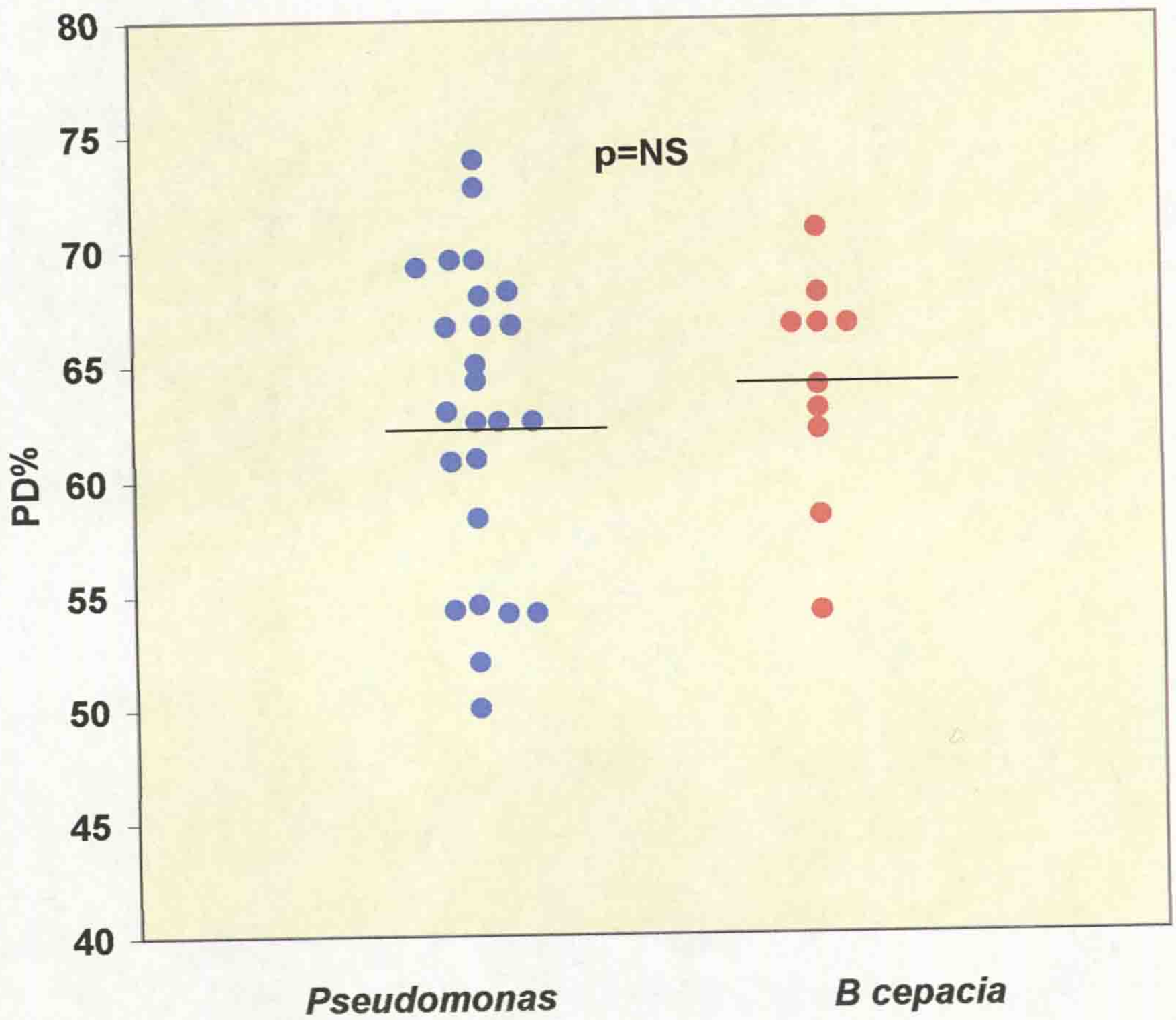


Table 4: Comparison between *Pseudomonas aeruginosa* (n = 25) and *B cepacia* (n=10) colonised patients

PARAMETER	MEAN VALUE FOR *Psa* PATIENTS (RANGE)	MEAN VALUE FOR *B cepacia* PATIENTS (RANGE)	P VALUE
PD%	62.79 (50-73.9)	64.03 (54.17-70.8)	0.552
Age (yrs)	24.4 (19-51)	24.8 (19-33)	0.866
FEV1 (% predicted)	56.2 (24-88)	44.9 (17-88)	0.247
FVC (% predicted)	73.1 (45-106)	59.8 (29-93)	0.234
O2 saturations (%)	97.2 (95-100)	95.1 (92-98)	0.050
Body mass index (kg/m^2)	20.2 (13.9-29.4)	20.5 (14.8-31.2)	0.866
Fasting glucose (mmol/l)	6.7 (4-22.4)	8.2 (4.3-24.8)	0.522
HbA1c (%)	6.3 (5.5-10.1)	6.7 (5.5-10.7)	0.504
Vitamin E (umol/l)	17.2 (4-38)	14.1 1-35	0.571
No of courses of iv antibiotics over previous 2 yrs	6.3 (1-17)	6.7 (1-11)	0.799
No of days on iv antibiotics over previous 2 yrs	97.9 (6-351)	109.2 (27-191)	0.666

Correlations between PD% and markers of disease severity in the whole patient group

None were found as indicated in Table 5.

Comparison between diabetic and non-diabetic patients

Patients were classified as diabetic if they were taking regular insulin. 11 diabetic and 24 non-diabetic patients took part. PD% did not differ significantly between the two groups (diabetics mean 64.59%, non-diabetics mean 62.48%, p=NS) (Fig 6). Only the mean values for number of courses and number of days of intravenous antibiotics over the preceding two years differed between the groups (diabetics mean 8.6 courses, non-diabetics mean 5.4 courses, p=0.042; diabetics mean 152.8 days, non-diabetics mean 76.6 days, p=0.019) (Figs 7 and 8 and Table 6).

There were no significant correlations between PD% and markers of disease severity for either of the 2 groups (Tables 7 and 8).

Table 5: Correlations between PD% and markers of disease severity in the patient group

CORRELATION	r VALUE	p VALUE
PD% vs FEV1	0.206	>0.1
PD% vs FVC	0.212	>0.1
PD% vs O_2 saturation	0.006	>0.1
PD% vs Body mass index	-0.076	>0.1
PD% vs Fasting glucose	0.091	>0.1
PD% vs HbA1c	0.104	>0.1
PD% vs Vitamin E	-0.142	>0.1
PD% vs no of courses of intravenous antibiotics	0.123	>0.1
PD% vs no of days on intravenous antibiotics	0.101	>0.1

Fig 6: Comparison of pupil diameter percent between diabetic (n=11) and non-diabetic (n=24) patients

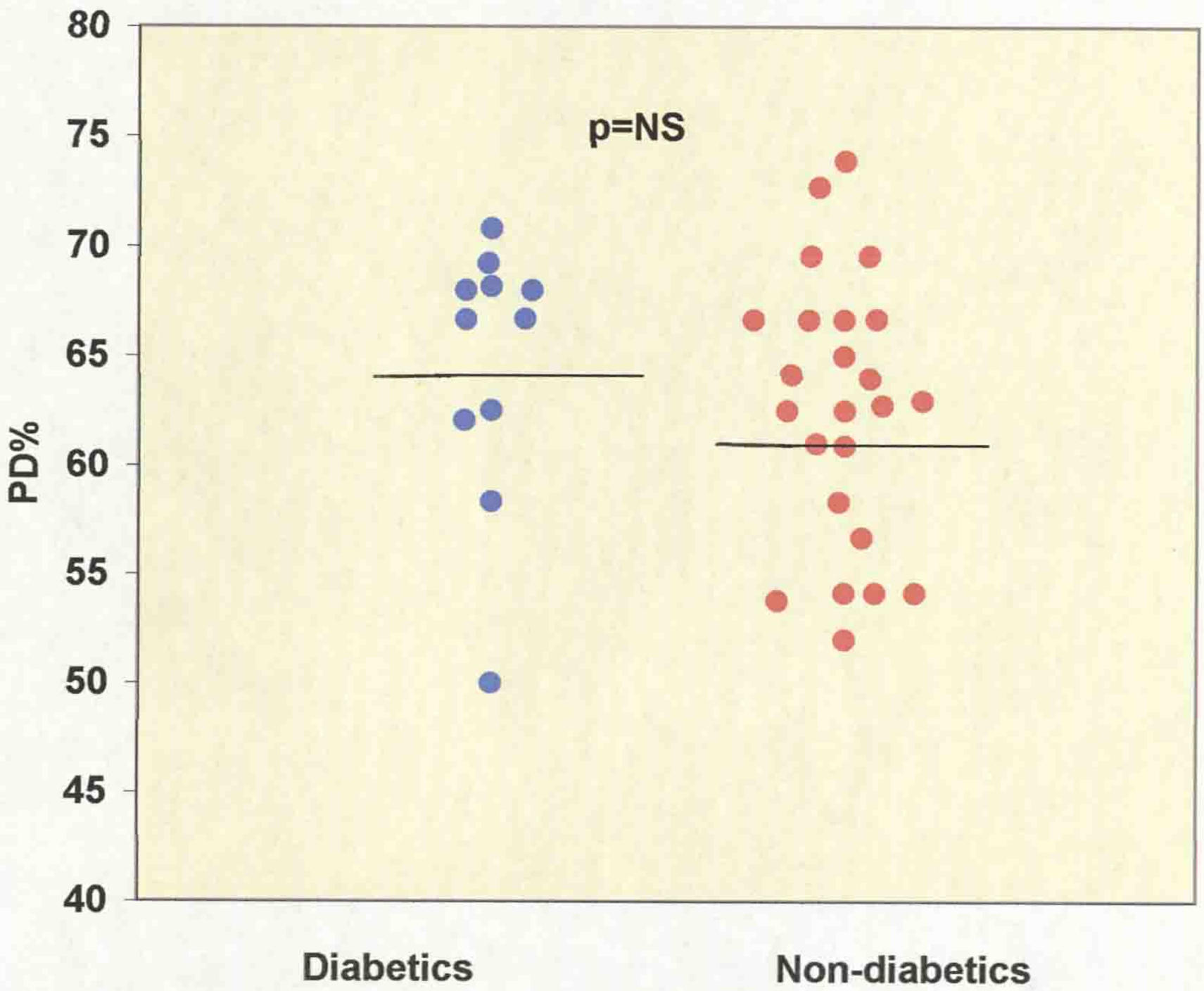
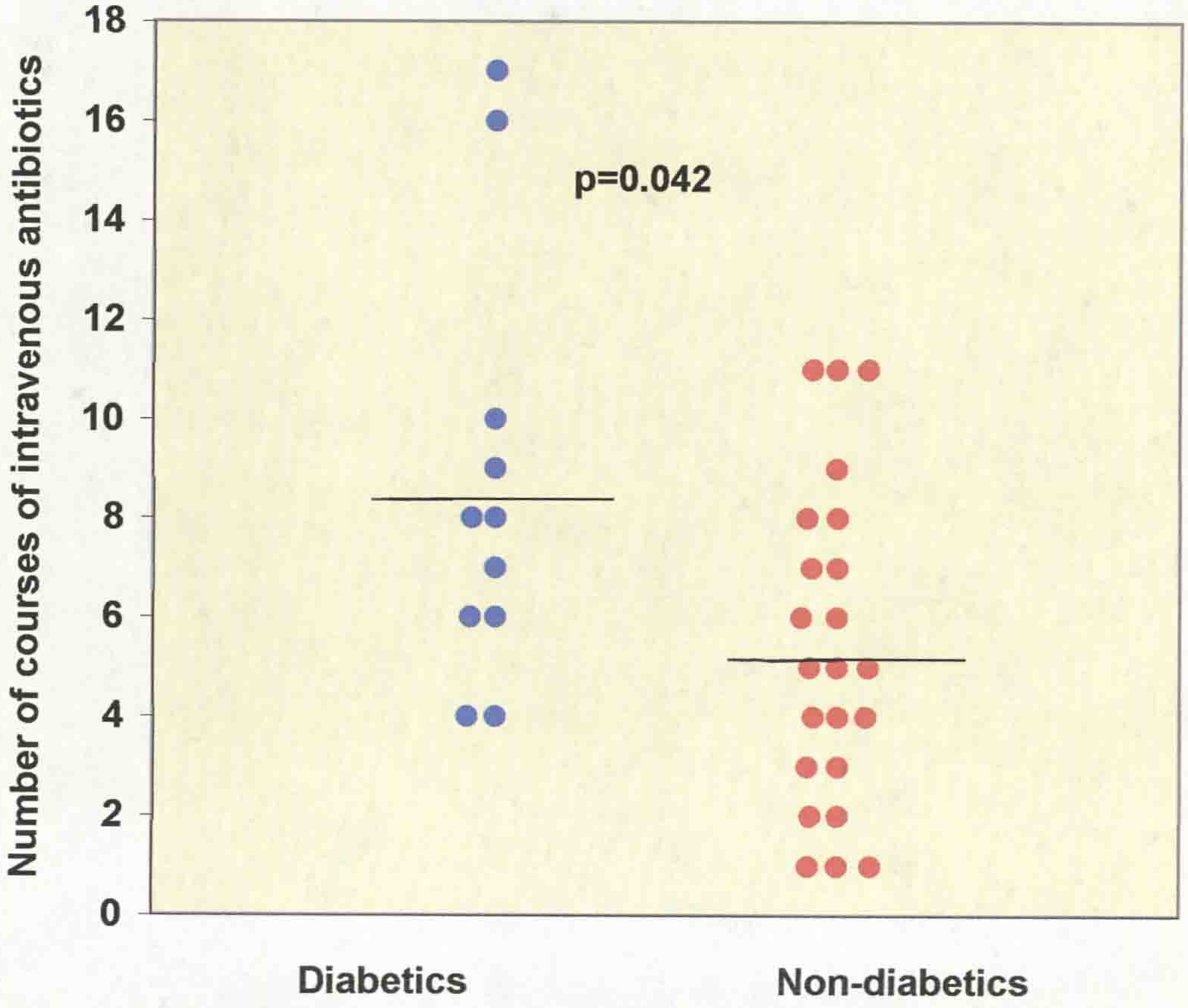
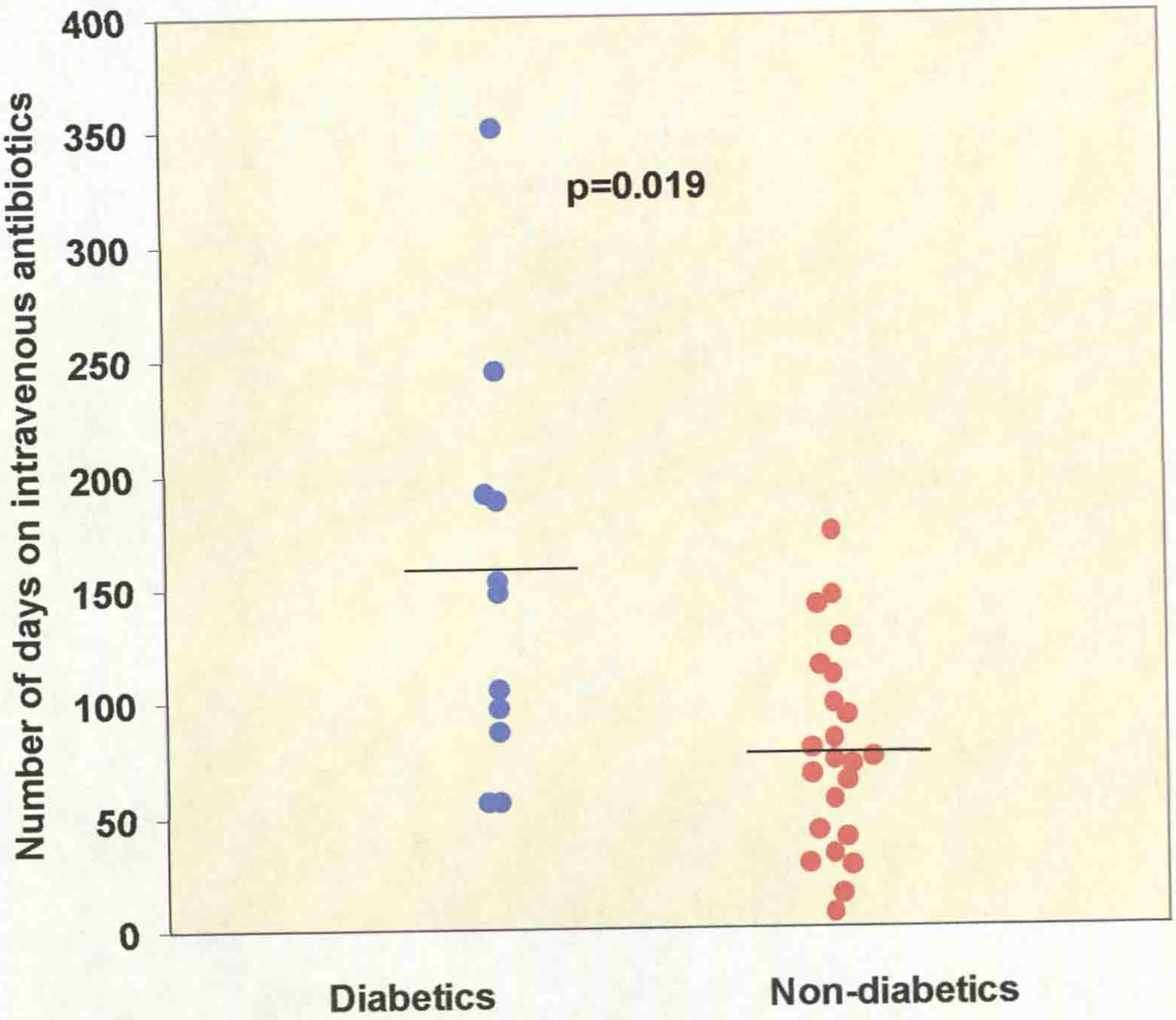


Fig 7: Comparison of number of courses of intravenous antibiotics over preceding 2 yrs in diabetic (n=11) and non-diabetic (n=24) patients



The number of courses of intravenous antibiotics was documented for only 23 non-diabetic patients.

Fig 8: Comparison of number of days on intravenous antibiotics over preceding 2 yrs in diabetic (n=11) and non-diabetic (n=24) patients



The number of days on intravenous antibiotics was documented for only 23 non-diabetic patients

Table 6: Comparison between diabetic (n = 11) and non-diabetic (n=24) patients

PARAMETER	MEAN VALUE FOR DIABETICS (RANGE)	MEAN VALUE FOR NON-DIABETICS (RANGE)	P VALUE
PD%	64.59 (50-70.8)	62.48 (52-73.9)	0.357
Age (yrs)	22.9 (19-29)	25.3 (19-51)	0.198
FEV1 (% predicted)	55.9 (24-88)	52.4 (17-88)	0.707
FVC (% predicted)	78.4 (45-106)	66.7 (29-95)	0.116
O2 saturations (% predicted)	97 (95-100)	96.6 (92-99)	0.522
Body mass index (kg/m^2)	19.9 (13.9-31.2)	20.5 14.8-29.4	0.697
Vitamin E (umol/l)	11.6 (1-38)	18.7 (4-37)	0.137
No of courses of iv antibiotics over previous 2 yrs	8.6 (4-17)	5.4 (1-11)	0.042
No of days on iv antibiotics over previous 2 yrs	152.8 (56-351)	76.6 (6-174)	0.019

Table 7: Correlations between PD% and markers of disease severity in diabetic CF patients (n=11)

CORRELATION	r VALUE	p VALUE
PD% vs FEV1	0.043	>0.1
PD% vs FVC	-0.047	>0.1
PD% vs O_2 saturation	0.367	>0.1
PD% vs Body mass index	-0.469	>0.1
PD% vs Fasting glucose	0.132	>0.1
PD% vs HbA1c	0.127	>0.1
PD% vs Vitamin E	0.227	>0.1
PD% vs no of courses of intravenous antibiotics	0.045	>0.1
PD% vs no of days on intravenous antibiotics	-0.037	>0.1

Table 8: Correlations between PD% and markers of disease severity in non-diabetic CF patients (n=24)

CORRELATION	r VALUE	p VALUE
PD% vs FEV1	0.262	>0.1
PD% vs FVC	0.217	>0.1
PD% vs O_2 saturation	-0.158	>0.1
PD% vs Body mass index	0.177	>0.1
PD% vs Fasting glucose	-0.302	>0.1
PD% vs HbA1c	-0.077	>0.1
PD% vs Vitamin E	-0.323	>0.1
PD% vs no of courses of intravenous antibiotics	0.051	>0.1
PD% vs no of days on intravenous antibiotics	0.031	>0.1

Summary of results

- My values of coefficients of variation for reproducibility and repeatability for PD% were 2.6% and 3.9% respectively, comparable to those found in the literature.

- The administration of 5mg salbutamol and 0.5mg ipratropium bromide in nebulised form through a facemask had no significant topical effect on pupil size.

- There was no significant difference in PD% between controls and CF patients, diabetics and non-diabetics, *Pseudomonas* and *B cepacia* colonised patients, or male and female patients.

5:2 OPHTHALMIC SYSTEM: DISCUSSION

Pupil diameter percent (PD%) is said to be one of the most reproducible tests of autonomic function (see below). Furthermore, measurement of PD% has previously been validated by comparison with the standard technique of infra-red television pupillometry; Smith and Dewhirst (1986) found a close correlation between PD% using a simple Polaroid photographic technique and pupil diameter measured with the TV method ($r=0.95$, $p<0.001$) in 15 insulin dependent diabetics.

Control subjects

My values for coefficients of variation of 2.6% and 3.9% for reproducibility and repeatability using a digital camera correlate well with values in the literature. Smith and Dewhirst (1986) found no difference in PD% between photographs taken 15 minutes apart in 10 control subjects; a value of 3.2% was obtained for the coefficient of variation when pupils were photographed 3 weeks apart in 20 controls. Similarly, Lanting et al (1990*a*) reported a coefficient of variation of PD% of 3.2% in controls whose eyes were photographed one week apart.

My data did not show any relationship between PD% and age. This is in contrast to previous studies. For example, Smith and Dewhirst (1986) demonstrated that PD% was strongly age-dependent ($p<0.001$) in 163 controls aged 16 to 92 years. Lanting et al (1990*a*) reported that PD% showed a significant decrease with ageing in 56 controls aged 12 to 75 years ($r=0.49$, $p<0.01$). Finally, Cahill et al (2001) also showed a decrease in mean dark adapted pupil diameter with increasing age in controls aged 5 to 87 years and in diabetics aged 4 to 79 years. However, Hreidarsson (1982), who examined pupil area with infrared TV-videopupillography in 109 insulin-dependent diabetics aged 24 to 43 years and in 39 controls aged 26 to 41 years, observed no correlation between age and pupil area in either group, in agreement with my data where the control group was aged 22 to 38 years. An explanation for this could be that both my study and that of Hreidarsson (1982) involved subjects with a much narrower age range than the other studies.

Effects of bronchodilators on pupil size

A double-blind, randomised, cross-over study by Watson et al (1994) looked at the effect of nebulised ipratropium bromide (an anticholinergic agent) and nebulised albuterol (a ß-agonist and sympathomimetic agent) on intraocular pressures and pupil size in children aged 6 to 17 years with asthma. Intraocular pressures and pupil size were measured before and 30 minutes after nebulisation with ipratropium bromide added to albuterol, albuterol alone, or saline solution given by facemask. In a subsequent open study, patients who had been admitted to hospital with acute asthma who were treated with nebulised ipratropium bromide were recruited for measurement of intraocular pressures and pupil size. 20 patients completed the double-blind study and 26 completed the open study. There were no changes in intraocular pressures or pupil size after any treatment on any study day in either the double blind or open study. Therefore, one can assume that in those patients with no pre-existing ocular abnormalities, the effects of ipratropium or albuterol delivered by nebuliser and facemask absorbed in the eye are negligible.

My data confirms that after administration of both nebulised bronchodilators through a facemask, the effects on PD% were negligible immediately after administration and at the end of a 30 minute period.

Patients

As with controls, PD% in CF patients did not correlate with age.

The difference in PD% between patients and control subjects was not statistically significant. Indeed, the lower limit of the range was identical in both groups (50%). However, it is possible that as in the cardiovascular system, parasympathetic dysfunction precedes sympathetic dysfunction, and therefore, if tests of parasympathetic function such as light reflex latency or pupil cycle time had been used, significant differences between controls and patients may have been observed. Evidence for this comes firstly from the work of Lanting et al (1990*a*), who studied diabetics with normal (Group 1) and abnormal (Group 2) heart rate responses to standing and deep breathing. 13 patients in Group 2 and 4 patients in Group 1 had a prolonged pupillary light reflex latency period (mean light reflex latency Group 2 266.7msec versus Group 1 228.8msec, $p<0.001$). PD% was abnormally small in only 7 patients in Group 2 and 1 in Group 1 (mean PD% Group 2 45.1% versus Group 1 57.9%, $p<0.01$). Furthermore, if the results of cardiovascular tests were taken as an indicator of whether or not autonomic neuropathy was present, the sensitivity of light reflex latency as a diagnostic test was 86.7% and specificity 73.3%, whereas the sensitivity of PD% was 46.7%, specificity 93.3%. More recently, Cahill et al (2001) reported that there was no significant difference in mean percentage dilatation in response to 4% cocaine (a catecholamine uptake inhibitor) in diabetics and controls. However, except for Type 1 diabetics with disease duration less than 5 years, all patient groups had significantly greater mean percentage constriction in pupil size in response to 0.1% pilocarpine in comparison to controls, indicating that damage to the pupillary parasympathetic pathway may precede pupillary sympathetic dysfunction.

Unfortunately, parasympathetic pupillary tests require the use of specialised equipment, which would have involved the transfer of the patients to other centres.

Another explanation for the lack of difference in PD% between patients and controls could be that despite its excellent reproducibility, PD% may be a relatively insensitive test for the detection of autonomic dysfunction. This has previously been suggested in the literature. For example, as mentioned above, Lanting et al (1990*a*) observed that the sensitivity of PD% was only 46.7% assuming that the results of cardiovascular autonomic function testing are an indication of whether or not autonomic neuropathy is present (although tests of parasympathetic function only were utilised). Interestingly, even if pupil cycle time (a parasympathetic test) was used as a diagnostic test, its sensitivity was still only 59% as reported by Martyn and Ewing (1986) in their study of 135 diabetics who also performed Ewing's battery of cardiovascular tests. However, it should be remembered that abnormalities in cardiovascular reflex tests do not necessarily reflect autonomic dysfunction in other systems. Smith and Dewhirst (1986) found that in 15 diabetics who performed cardiovascular tests, PD% weakly correlated with only postural change in systolic blood pressure ($r=0.56$, $p<0.05$) and the mean difference in RR interval length during inspiratory and expiratory phases of 5 successive deep breaths ($r=0.59$, $p<0.05$), but not with the Valsalva ratio ($r=0.446$, $p=NS$) or the standard deviation of 255 successive RR intervals ($r=0.29$, $p=NS$). Finally, although in a study of 142 adolescents with diabetes, Schwingshandl et al (1993) observed that mean resting pupil diameters were significantly smaller in comparison to controls, no correlation was found between pupil diameter and cardiovascular reflex test results when considered as a group or when separated into single tests.

Pharmacological pupil tests have been utilised to assess autonomic function in CF patients; Davis et al (1980) showed that CF patients had pupillary dilatation of at least 0.5mm in response to significantly lower concentrations of topical phenylephrine compared to controls (CF mean phenylephrine concentration 1.36% versus controls 2.67%, $p<0.0001$). Furthermore, CF patients demonstrated pupillary constriction of at least 1mm in the dark in response to significantly lower concentrations of topical carbachol (CF mean carbachol concentration 0.46% versus controls 0.82%, $p<0.0001$). However, although pharmacological pupil function tests are a useful supplement to the measurement of pupil cycle time, light reflex latency, PD% and redilatation time, they have disadvantages. For example, there is poor bioavailability from eyedrops due to

low and variable corneal penetration particularly in dark eyes with more melanin pigment. In addition, the effects of drugs on the pupil can last up to 48 hours (Smith, 1992). For these reasons, pharmacological tests were not used in CF patients in this study.

Comparison between *B cepacia* and *Pseudomonas* colonised patients

Colonisation with *B cepacia* leads to an increase in morbidity and deterioration of pulmonary function compared to *Pseudomonas aeruginosa* colonisation. In some cases high fever, progressive respiratory failure and elevated inflammatory markers with a leucocytosis can occur associated with a high fatality rate (Isles et al, 1984). In addition, Ledson et al (1998) demonstrated that epidemic strains could cross-colonise CF patients already infected with *B cepacia*, with fatal consequences. Therefore, it was expected that *B cepacia* colonised patients might have worse autonomic function and lower PD% values compared to *Pseudomonas* colonised patients. However, PD% did not differ significantly between the 2 groups, although if tests of parasympathetic function had been used, a difference may have been revealed.

Comparison between diabetic and non-diabetic patients

46% of patients in my study were diabetic (on the basis of regular insulin requirement). As diabetes is a major factor contributing towards autonomic neuropathy, it was expected that PD% would differ significantly between the 2 groups. This was not the case. Again, had parasympathetic tests been used, a difference may have been seen. However, it should be noted that cystic fibrosis related diabetes mellitus (CFRDM) is distinct from conventional Type I and Type II diabetes. Unlike Type I, CFRDM is of slower onset (often a prodromal period of 2 years or more), ketosis is not a feature and some insulin secretion persists. Similarly, unlike Type II, CFRDM patients are often underweight (rather than obese). There is also an associated pancreatic exocrine defect (Hodson, 1992).

Several investigators have reported the occurrence of diabetic complications in CF patients. For example, Rodman et al (1986) described 2 out of 24 patients with CFRDM who developed retinopathy (one with macular oedema, one with multiple microaneursyms). Sullivan and Denning (1989) reported that 4 out of 19 patients with CFRDM had evidence of microangiopathy. In one, peripheral neuropathy developed 5 years after the onset of diabetes mellitus, and the other 3 patients each had complications of retinopathy, nephropathy and neuropathy which developed 10 years after the onset of diabetes. All were poorly compliant in their medical care. Finally, Lanng et al (1994) identified 4 out of 41 CFRDM patients who developed background retinopathy (2 patients), nephropathy (1 patient), microalbuminuria (1 patient) and neuropathy (2 patients). However, the overall incidence of diabetic complications in CF is probably quite low as most patients still probably do not live long enough to develop microvascular complications, which may also explain the lack of a significant difference in PD% between diabetic and non-diabetic patients in my study. In my study, only the mean values for the number of courses and number of days of intravenous antibiotics over the preceding 2 years differed significantly between diabetics and non-diabetics. It may be that the diabetic patients suffered more pulmonary infections requiring more antibiotic therapy in comparison to controls; it is certainly known that insulin deficiency in cystic fibrosis patients can lead to a decline in pulmonary function (Rosenecker et al, 2001) which could in turn increase the requirement for antibiotic treatment in this group.

Correlations with markers of disease severity

There have been no previous studies of CF patients looking at the relationship between pupillary autonomic function and pulmonary function, nutritional state, glycaemic control, vitamin E levels or the requirement for antibiotic therapy.

My data shows that there was no correlation between PD% and lung function. As before, if parasympathetic tests had been used then significant correlations may have been revealed. Furthermore, data from the cardiovascular spectral analysis section of my study has shown no significant difference in low frequency power (sympathetic function) and controls ($p=0.20$), whereas there was a significant difference in high frequency power ($p=0.004$).

There was no correlation of PD% with nutritional state. Casu et al (2002) reported that in 13 patients with anorexia nervosa (mean body mass index 16.9), although the change in high frequency power (parasympathetic function) during an orthostatic load was significantly reduced in comparison to controls (with normal body mass index), there was no significant difference in the change in low frequency power in (sympathetic function) in comparison to controls, indicating that in nutritionally deficient patients, parasympathetic dysfunction precedes sympathetic dysfunction possibly accounting for the lack of correlation between PD% and body mass index in my patient group.

My data failed to show any relationship between PD% and fasting glucose levels or HbA1c. Previous work on diabetes, has produced conflicting results with respect to the correlation between pupil size and duration and degree of control of diabetes. For example, Smith et al (1978) found no correlation between pupil diameter and the mean of three previous clinic estimations of blood glucose concentrations in 36 insulin dependent diabetics. However, Hreidarsson (1982) in a study of 109 insulin dependent diabetics demonstrated an inverse relationship between diabetes duration and pupil area ($r=-0.33$, $p<0.0001$). Furthermore, in the group of long-term diabetics (duration greater than 15 years), non-fasting blood glucose levels correlated negatively with pupil area ($r=-0.49$, $p<0.0001$). Schwingshandl et al (1993) also reported significantly smaller resting pupil diameters with longer duration of diabetes ($r=-0.29$, $p<0.0001$), higher levels of

glycosylated haemoglobin (r=-0.24, p=0.004) and higher random blood glucose concentrations (r=-0.19, p=0.03) in 142 adolescents with Type I diabetes. However, random blood glucose measurements are unreliable in the diagnosis of CFRDM. Yung et al (1999) in their study of 91 CF patients reported that an abnormal random blood glucose value (greater than 11.1mmol/l) when used alone had a poor sensitivity in the diagnosis of CFRDM. This is not unexpected as it is known that basal insulin levels are relatively well preserved in CFRDM (Cucinotta et al, 1996). Increased levels of HbA1c also do not reliably identify CFRDM. Lanng et al (1995) performed a 5 year prospective study looking at the prevalence and incidence of diabetes in CF patients using the annual oral glucose tolerance test as the gold standard method of identification. At diagnosis of diabetes, increased HbA1c levels (greater than 6.4%) were seen in only 16% of patients.

The effects of vitamin E deficiency on neurological function in CF have been well documented. Sitrin et al (1987) reported abnormal eye movements, diminished reflexes and decreased vibratory and position sense in 2 CF patients with severe vitamin E deficiency. Cynamon et al (1988) demonstrated increased sural nerve conduction latencies and decreased nerve action potentials in 8 CF patients with vitamin E deficiency. However, the effects on autonomic nerve function have not previously been investigated. My data has shown no correlation between vitamin E levels and PD%; a significant number of patients (45%) had vitamin E levels in the normal range and hence were not deficient anyway.

Lastly, there was no relationship between PD% and the requirement for antibiotic therapy. It was postulated that the greater the requirement, the sicker the patient and the greater the likelihood of autonomic nerve damage. This, however, may affect parasympathetic before sympathetic function.

CHAPTER 6: GASTROINTESTINAL SYSTEM/ BOWEL SOUNDS (I)

6:1 SPECTRAL ANALYSIS: RESULTS

6:2 SPECTRAL ANALYSIS: DISCUSSION

6:1 SPECTRAL ANALYSIS: RESULTS

Control subjects

18 control subjects participated in the study, 1 male and 17 female. Their mean age was 28.5 years, range 22 to 35 years. The mean power was –23.9 dB, range -28.1 to -7.2 dB. The mean value for median frequency was 455.3Hz, range 226.6 to 515.6Hz. There was no correlation with age for either of these parameters (mean power: r=-0.029, p=NS; median frequency: r=0.172, p= NS).

Repeatability study

2 control subjects underwent testing on 3 consecutive days. Mean values, standard deviations and coefficents of variation for median frequency and mean power are shown in Tables 1a and 1b. There are no previous values for these in the literature.

Table 1a: Repeatability study for median frequency (MF) in 2 control subjects

SUBJECT	MF_{day1} (Hz)	MF_{day2} (Hz)	MF_{day3} (Hz)	MEAN MF (Hz)	SD	CV(%)
1	484.4	492.2	460.9	479.17	16.29	3.4
2	382.8	414.1	492.2	429.7	56.34	13.11

Mean CV = 8.25%

Table 1b: Repeatability study for mean power (MP) in 2 control subjects

SUBJECT	MP_{day1} (Hz)	MP_{day2} (Hz)	MP_{day3} (Hz)	MEAN MP (Hz)	SD	CV(%)
1	-36	-38.8	-40.1	-38.3	2.09	5.47
2	-35.2	-39.2	-36.7	-37.03	2.02	5.46

Mean CV = 5.465%

Patients

There were 16 CF patients, 6 male and 10 female. The mean age was 23.7 years, range 19 to 42 years. Mean values for power and median frequency were –22.7dB (range –27.3 to –18.4dB) and 434.6Hz (range 273.4 to 523.4Hz) respectively. Again there was no correlation with age (mean power: r=-0.126, p=NS; median frequency: r=0.062, p=NS).

Table 2 indicates mean values (plus ranges) for markers of disease severity in the patient group.

Patients with constipation

7 of the above 16 patients had constipation at the time of the recordings, 1 male and 6 female. The mean age was 21.9 years, range 19 to 29 years. Mean values for power and median frequency were –22.5dB (range –27.3 to –18.4dB) and 433.0Hz (range 273.4 to 500Hz) respectively.

Table 3 illustrates mean values (plus ranges) for markers of disease severity in these patients.

Patients without constipation

9 patients were included, 5 male and 4 female. Their mean age was 24.7 years, range 19 to 42 years. The values for mean power and median frequency were –23.0dB (range -26.5 to –18.5dB) and 435.8Hz (range 289.1 to 523.4Hz) respectively.

Mean values for markers of disease severity are indicated in Table 4.

Table 2: Mean values for markers of disease severity in the CF patient group (n=16)

PARAMETER	MEAN VALUE (RANGE)
Age (years)	23.7 (19-42)
FEV1 (% predicted)	64.2 (24-88)
FVC (% predicted)	79.4 (45-106)
O2 saturations (%)	97.3 (95-100)
BMI (kg/m^2)	20.2 (13.9-23.7)
Fasting glucose (mmol/l)	8.8 (4.3-22.4)
HbA1c (%)	6.6 (5.5-10.1)
Vitamin E (umol/l)	12.2 (4-23)
No of courses of iv antibiotics over previous 2 yrs	6 (1-16)
No of days on iv antibiotics over previous 2 years	91.2 (14-245)

Table 3: Mean values for markers of disease severity in constipated CF patients (n = 7)

PARAMETER	MEAN VALUE (RANGE)
Age (years)	21.9 (19-29)
FEV1 (% predicted)	79.2 (64-88)
FVC (% predicted)	86.2 (83-92)
O2 saturations (%)	98.3 (97-100)
BMI (kg/m^2)	20.9 (18.1-23.7)
Fasting glucose (mmol/l)	8.7 (4.3-16.5)
HbA1c (%)	6.2 (5.5-6.9)
Vitamin E (umol/l)	13.4 (4-23)
No of courses of iv antibiotics over previous 2 years	7.3 (4-14)
No of days on iv antibiotics over previous 2 years	107 (56-188)

Table 4: Mean values for markers of disease severity in non-constipated patients (n = 9)

PARAMETER	MEAN VALUE (RANGE)
Age (years)	25.2 (19-42)
FEV1 (% predicted)	53 (24-82)
FVC (% predicted)	74.4 (45-106)
O2 saturations (%)	96.5 (95-98)
BMI (kg/m^2)	19.4 (13.9-23)
Fasting glucose (mmol/l)	8.9 (4.3-22.4)
HbA1c (%)	6.9 (5.7-10.1)
Vitamin E (umol/l)	11.3 (4-19)
No of course of iv antibiotics over previous 2 yrs	4.9 (1-16)
No of days on iv antibiotics over previous 2 yrs	77.4 (14-245)

Comparison between all CF patients and control subjects

There were no significant differences between the two groups for either mean power (CF patients –22.7dB, controls –23.9dB, p=NS) (Fig 1) or median frequency (CF patients 434.6Hz, controls 455.3Hz, p=NS) (Fig 2).

Comparison between diabetic and non-diabetic patients

Patients were classified as diabetic on the basis of regular insulin requirement. 7 diabetic and 9 non-diabetic patients took part. The median frequency was significantly different between the groups (diabetics 481Hz, non-diabetics 398.5Hz, p=0.018) (Fig 3) but not the mean power or markers of disease severity (Table 5).

Comparison between non-constipated CF patients and control subjects

Again, this revealed no significant differences for mean power (CF patients –22.9dB, controls –23.9dB, p=NS) (Fig 4) or median frequency (CF patients 435.8Hz, controls 455.3Hz, p=NS) (Fig 5).

Comparison between constipated CF patients and control subjects

Mean values for mean power were: CF patients –22.5dB versus controls –23.9dB, p=NS (Fig 6). Likewise for median frequency, mean values were: CF patients 433.0Hz, controls 455.3Hz, p=NS (Fig 7).

Comparison between constipated and non-constipated CF patients

There were no significant differences for mean power (constipated patients –22.5dB versus non-constipated patients –22.9dB, p=NS) (Fig 8) or median frequency (constipated patients 433.0Hz versus non-constipated patients 435.8Hz, p=NS) (Fig 9). Table 6 shows markers of disease severity in these 2 groups.

Fig 1: Comparison of mean power between controls (n=18) and all CF patients (n=16)

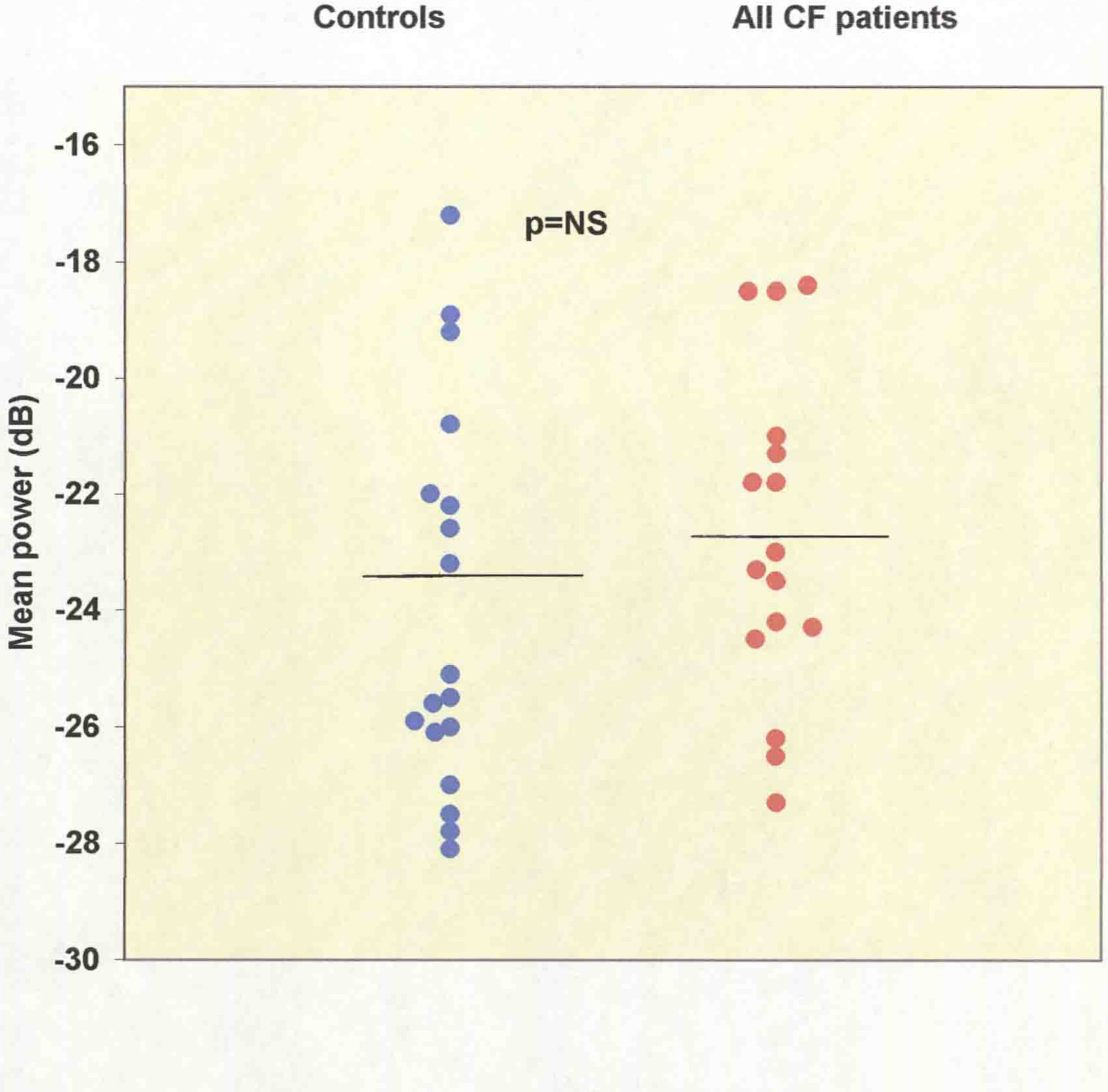


Fig 2: Comparison of median frequency between controls (n=18) and all CF patients (n=16)

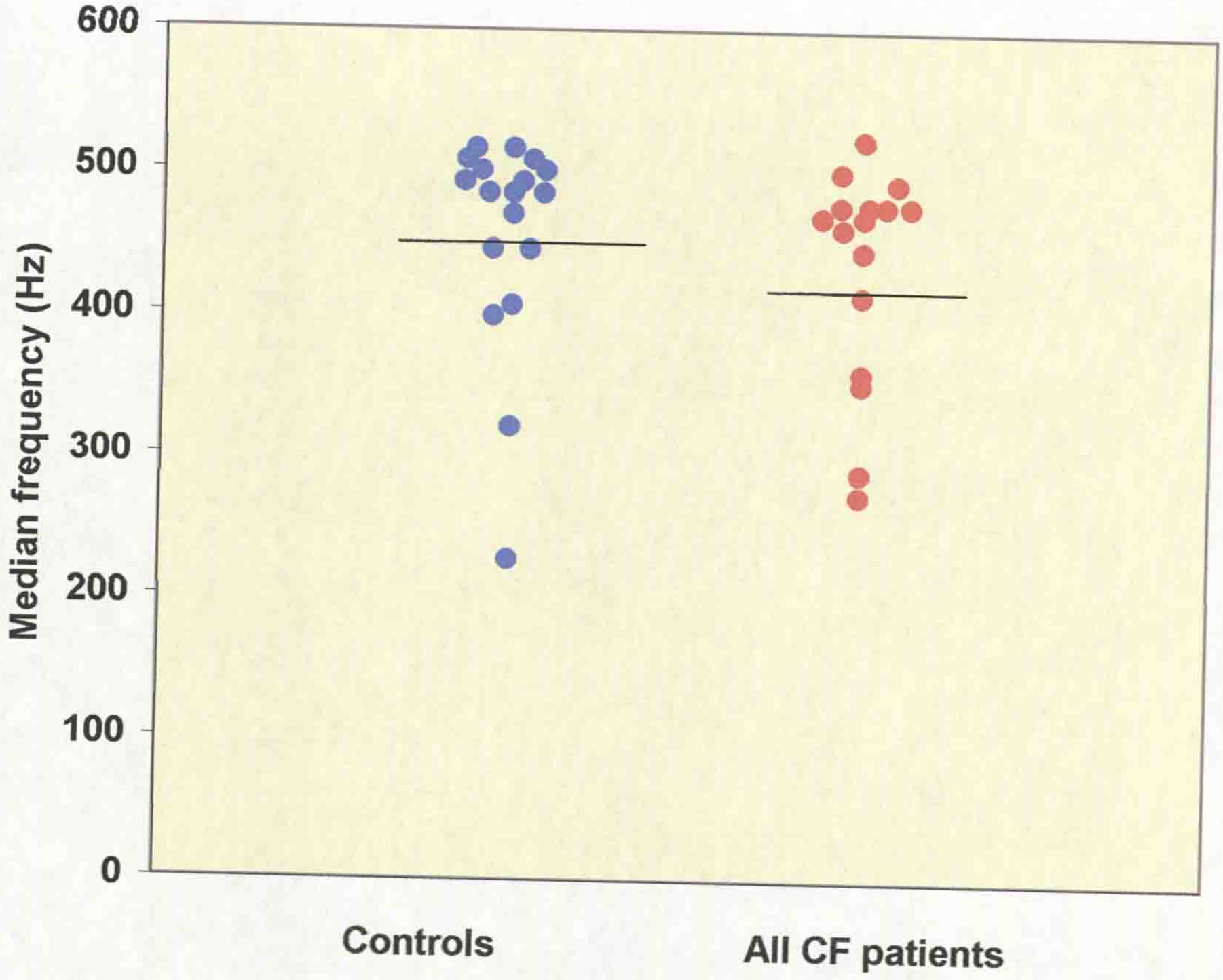


Fig 3: Comparison of median frequency between diabetic (n=7) and non-diabetic CF patients (n=9)

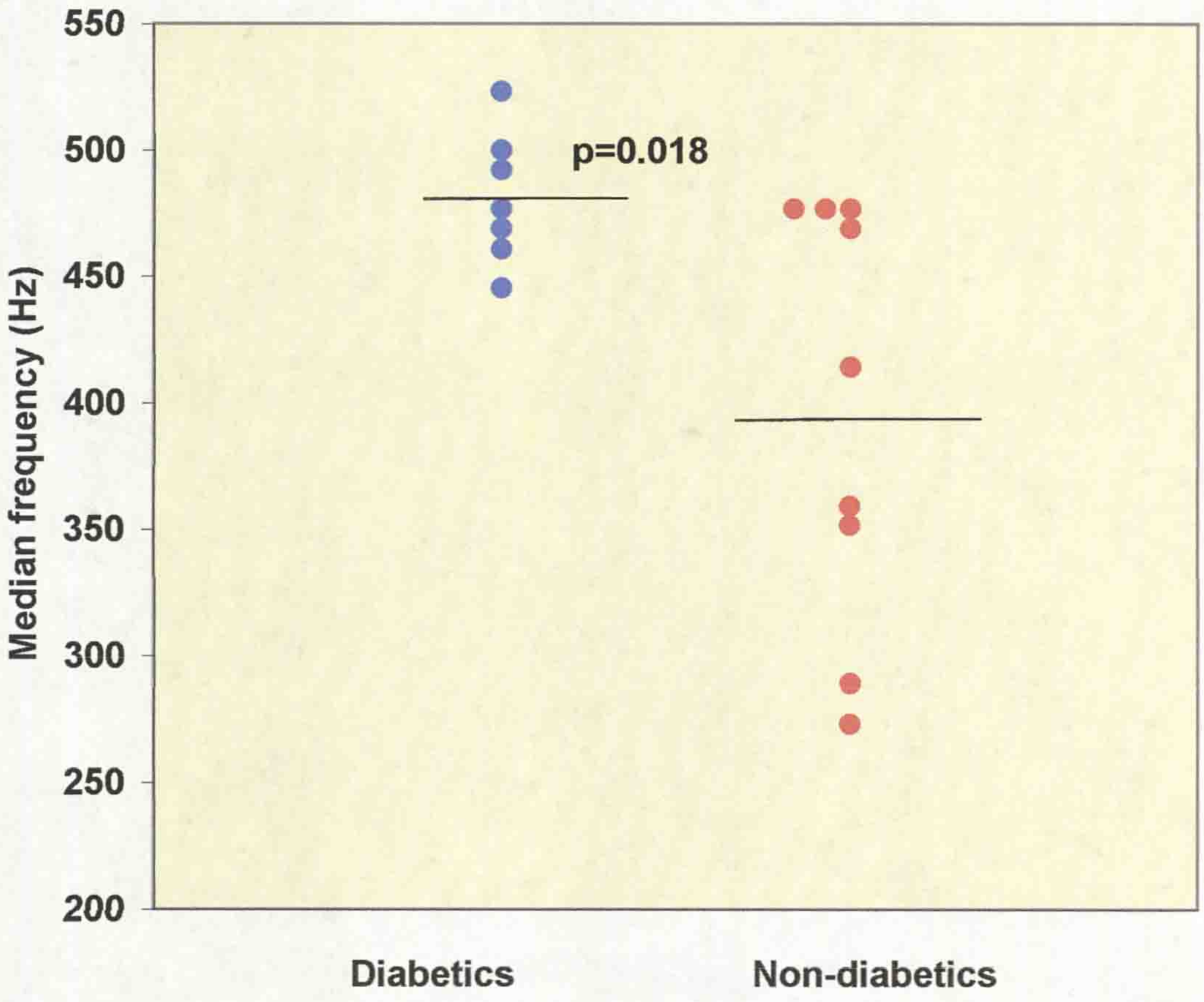


Table 5: Comparison between diabetic (n = 7) and non-diabetic patients (n=9)

PARAMETER	MEAN VALUE (DIABETICS) (RANGE)	MEAN VALUE (NON-DIABETICS) (RANGE)	P VALUE
Median frequency (Hz)	481.0 (445.3-523.4)	398.5 (273.4-476.6)	0.018
Mean power (dB)	-23.1 (-27.3-[-18.5])	-22.5 (-26.2-[-18.4])	0.710
Age (yrs)	25.6 (19-42)	22 (19-27)	0.300
FEV1 (% predicted)	63.5 (24-88)	64.8 (31-82)	0.924
FVC (% predicted)	86 (45-106)	74.5 (49-85)	0.281
O2 saturations (%)	97.5 (96-100)	97.1 (95-99)	0.651
BMI (kg/m^2)	19.6 (13.9-23.7)	20.7 (17.8-23)	0.442
Vitamin E (umol/l)	12 (4-23)	12.3 (4-19)	0.932
No of courses of iv antibiotics over previous 2 yrs	7.1 (4-16)	5 (1-14)	0.359
No of days of iv antibiotics over previous 2 yrs	115.8 (56-245)	69.6 (14-171)	0.190

Fig 4: Comparison of mean power between controls (n=18) and non-constipated CF patients (n=9)

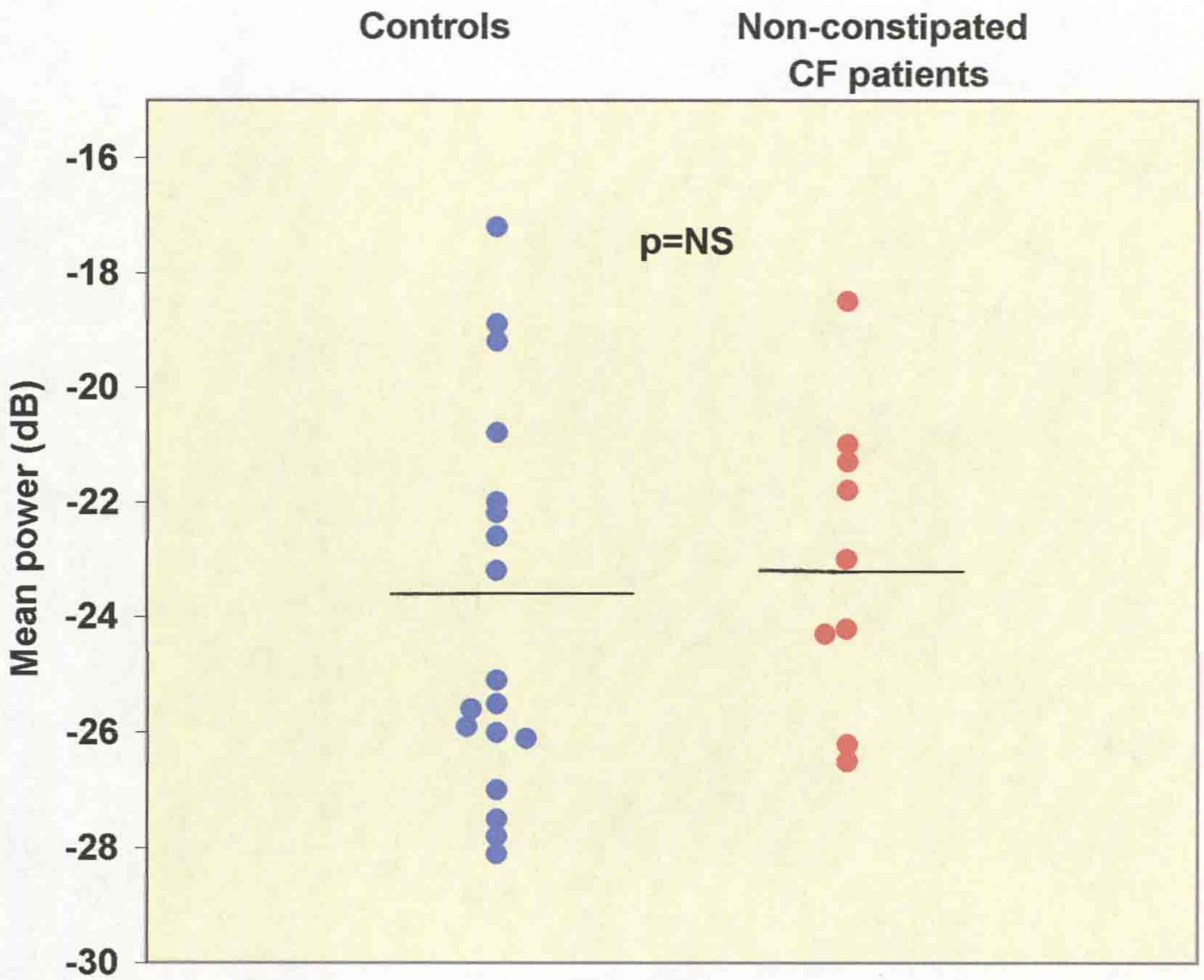


Fig 5: Comparison of median frequency between controls (n=18) and non-constipated CF patients (n=9)

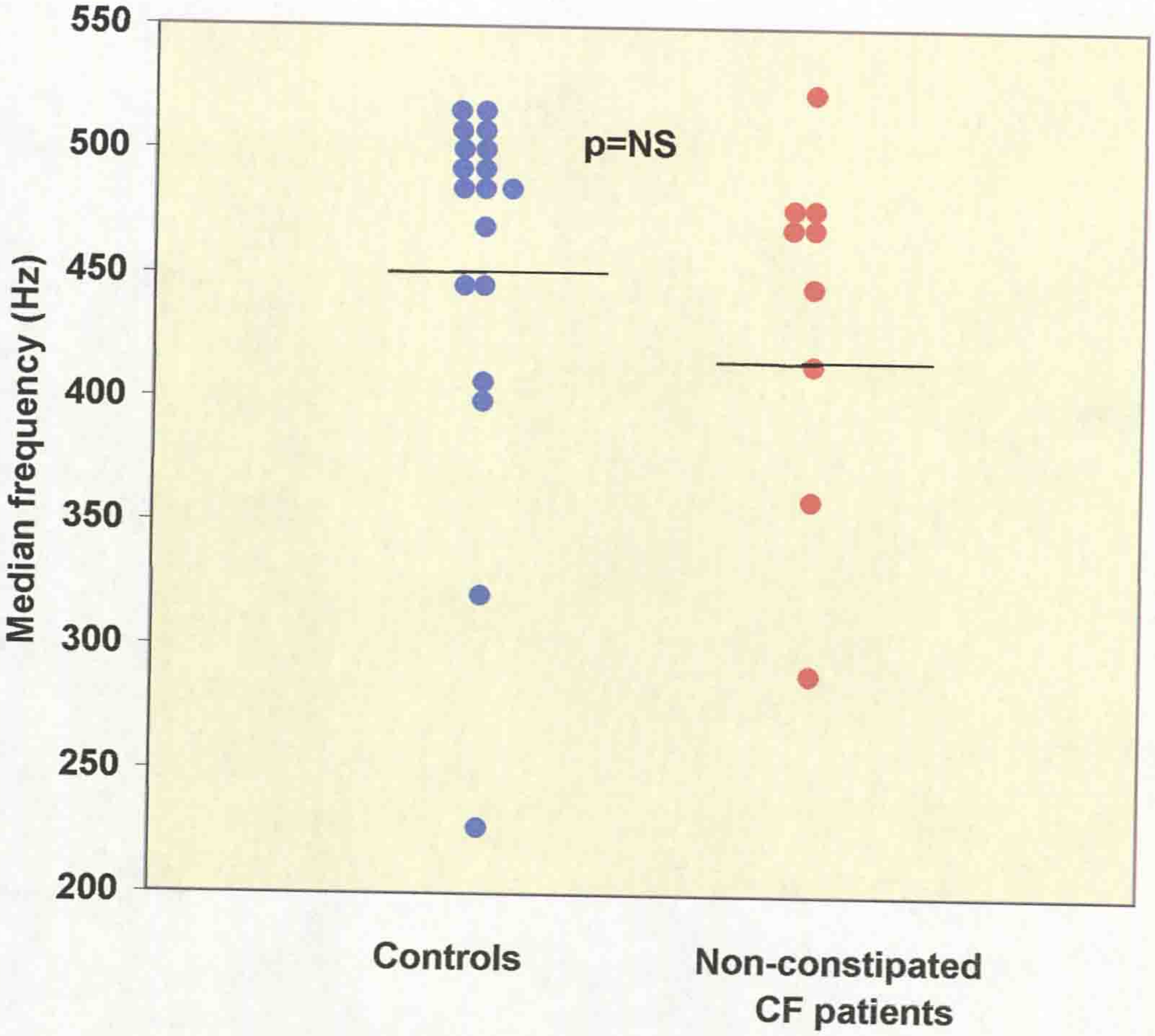


Fig 6: Comparison of mean power between controls (n=18) and constipated CF patients (n=7)

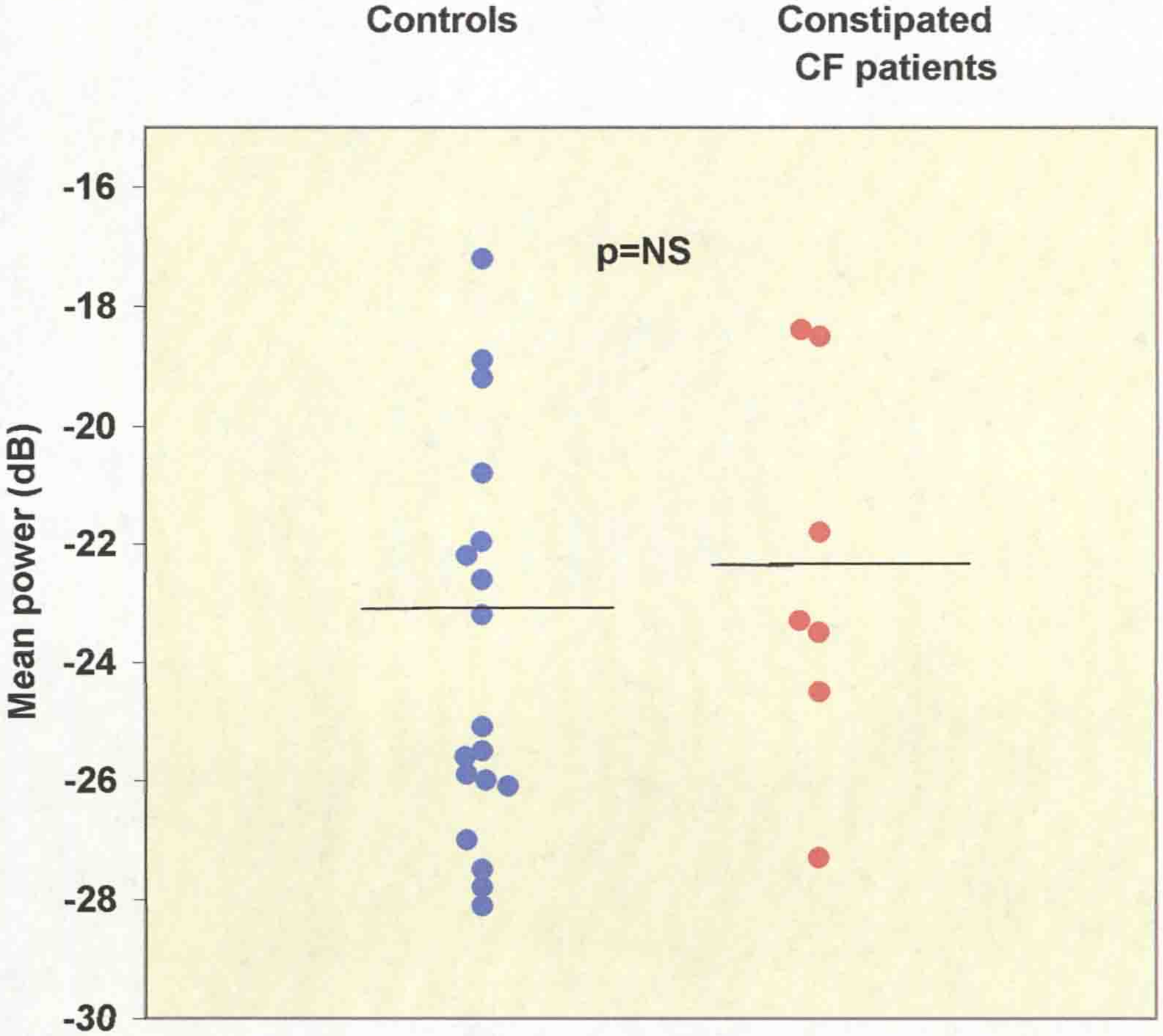


Fig 7: Comparison of median frequency between controls (n=18) and constipated CF patients (n=7)

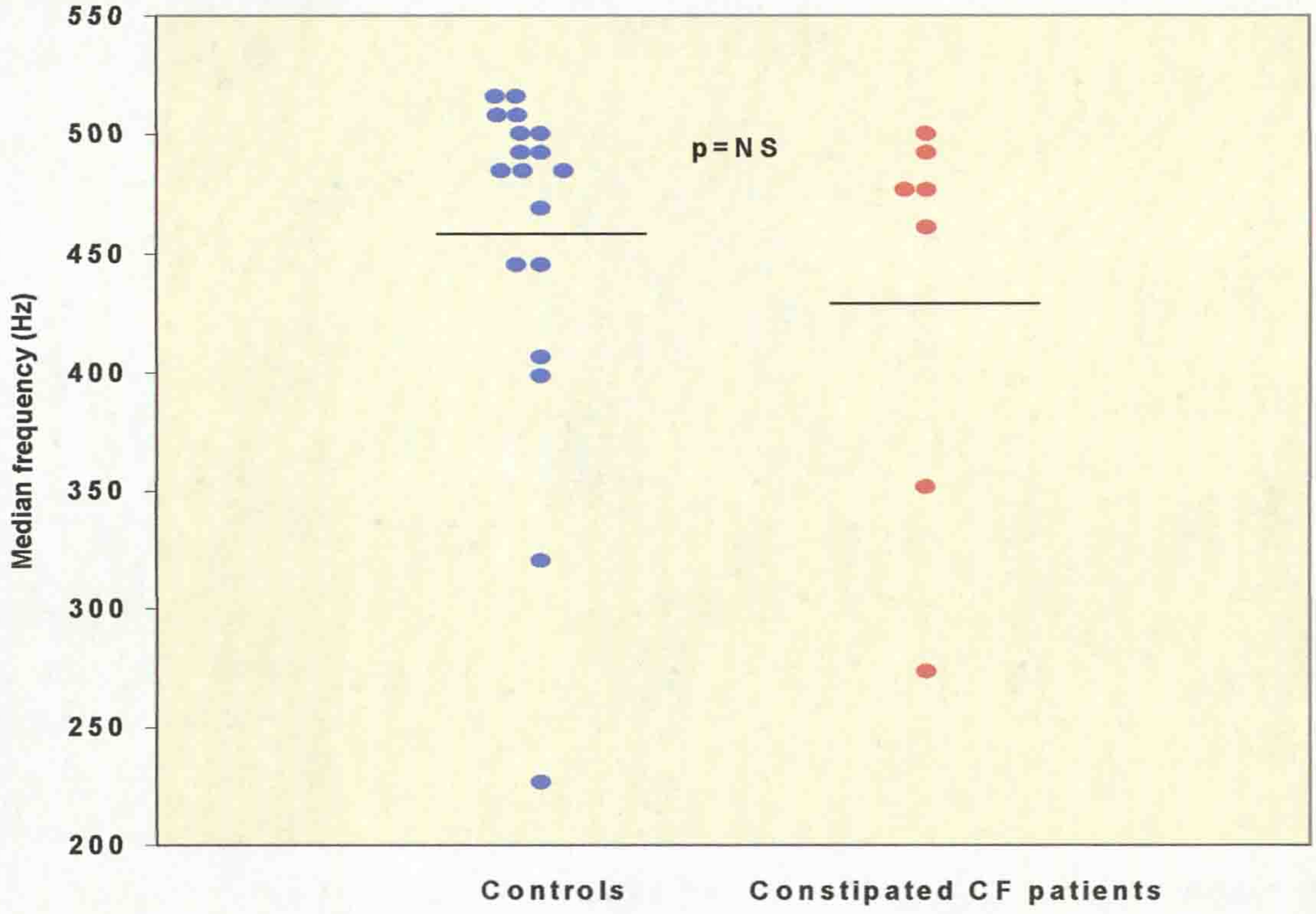


Fig 8: Comparison of mean power between constipated (n=7) and non-constipated CF patients (n=9)

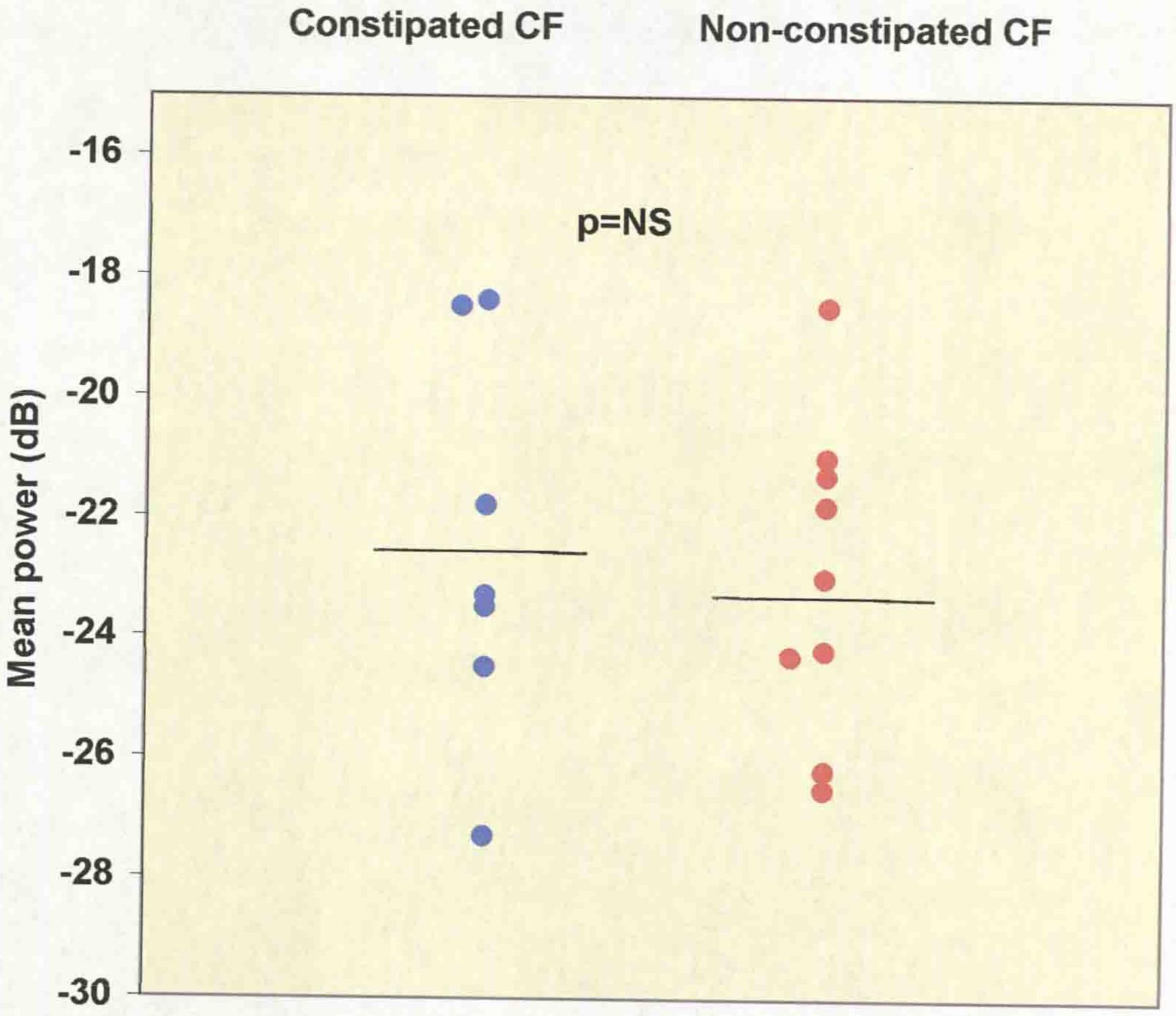


Fig 9: Comparison of median frequency between constipated (n=7) non-constipated CF patients (n=9)

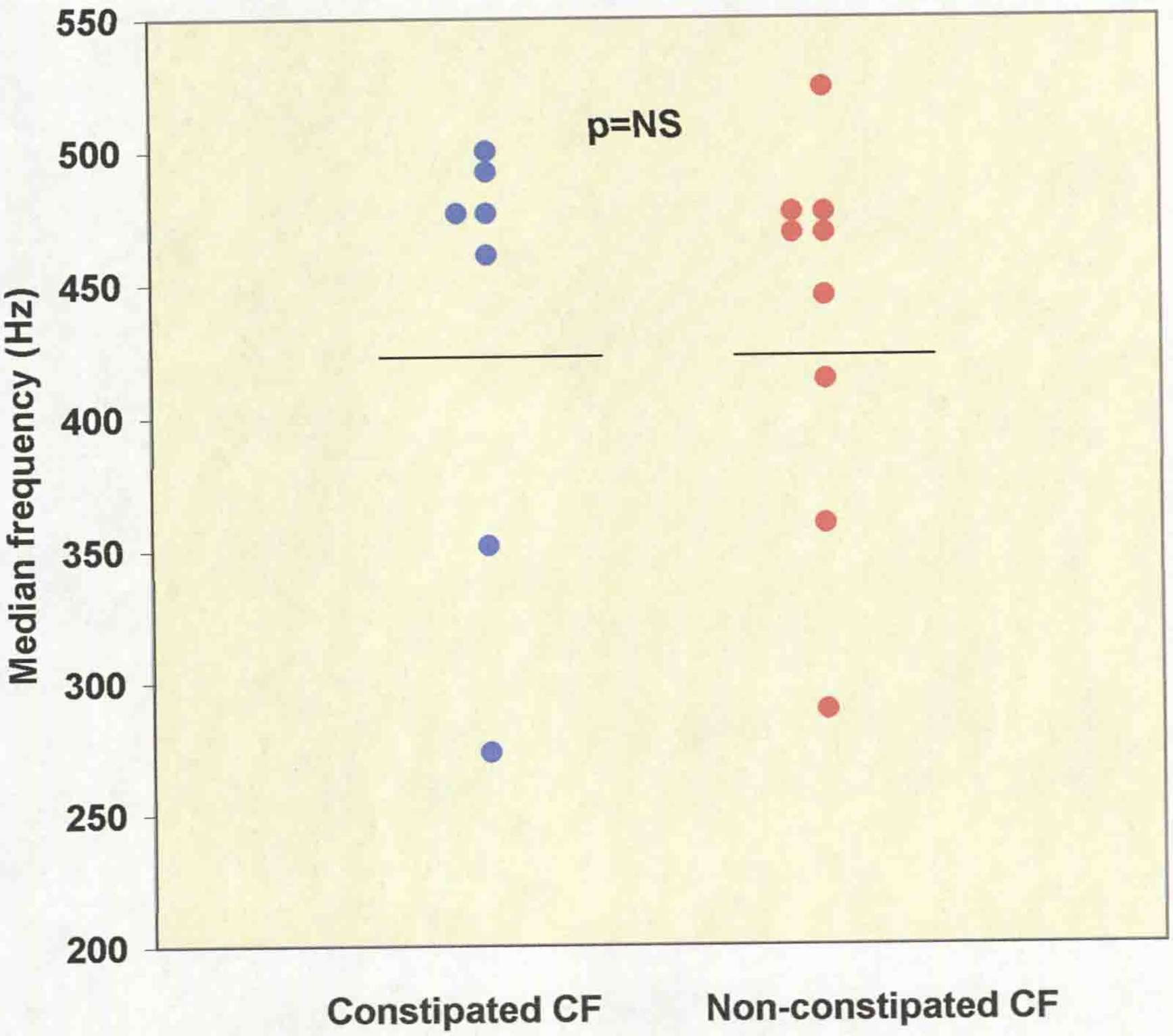


Table 6: Comparison between markers of disease severity for constipated (n=7) and non-constipated CF patients (n=9)

PARAMETER	MEAN VALUE (CONSTIPATED) (RANGE)	MEAN VALUE (NON-CONSTIPATED) (RANGE)	P VALUE
Age (years)	21.9 (19-29)	25.2 (19-42)	0.269
FEV1 (% predicted)	79.2 (64-88)	53 (24-82)	0.011
FVC (% predicted)	86.2 (83-92)	74.4 (45-106)	0.175
O2 saturations (%)	98.3 (97-100)	96.5 (95-98)	0.01
BMI (kg/m^2)	20.9 (18.1-23.7)	19.4 (13.9-23)	0.274
Fasting glucose (mmol/l)	8.7 (4.3-16.5)	8.9 (4.3-22.4)	0.934
HbA1c (%)	6.2 (5.5-6.9)	6.9 (5.7-10.1)	0.211
Vitamin E (umol/l)	13.4 (4-23)	11.3 (4-19)	0.618
No of course of iv antibiotics over previous 2 yrs	7.3 (4-14)	4.9 (1-16)	0.290
No of days on iv antibiotics over previous 2 yrs	107 (56-188)	77.4 (14-245)	0.386

Correlations with markers of disease severity

Only the mean power in the whole CF group correlated with number of courses ($r=0.527$, $p<0.05$) and number of days on intravenous antibiotics over the previous 2 years ($r=0.567$, $p<0.05$) (Figs 10 and 11 respectively).

For diabetic patients, mean power also correlated with these markers of disease severity ($r=0.854$, $p<0.01$ and $r=0.908$, $p<0.01$ respectively) (figs 12 and 13). There were no correlations in the non-diabetic patients.

Fig 10: **Correlation between mean power and number of courses of intravenous antibiotics over previous 2 years for 15 CF patients; information on number of courses was unavailable for 1 patient**

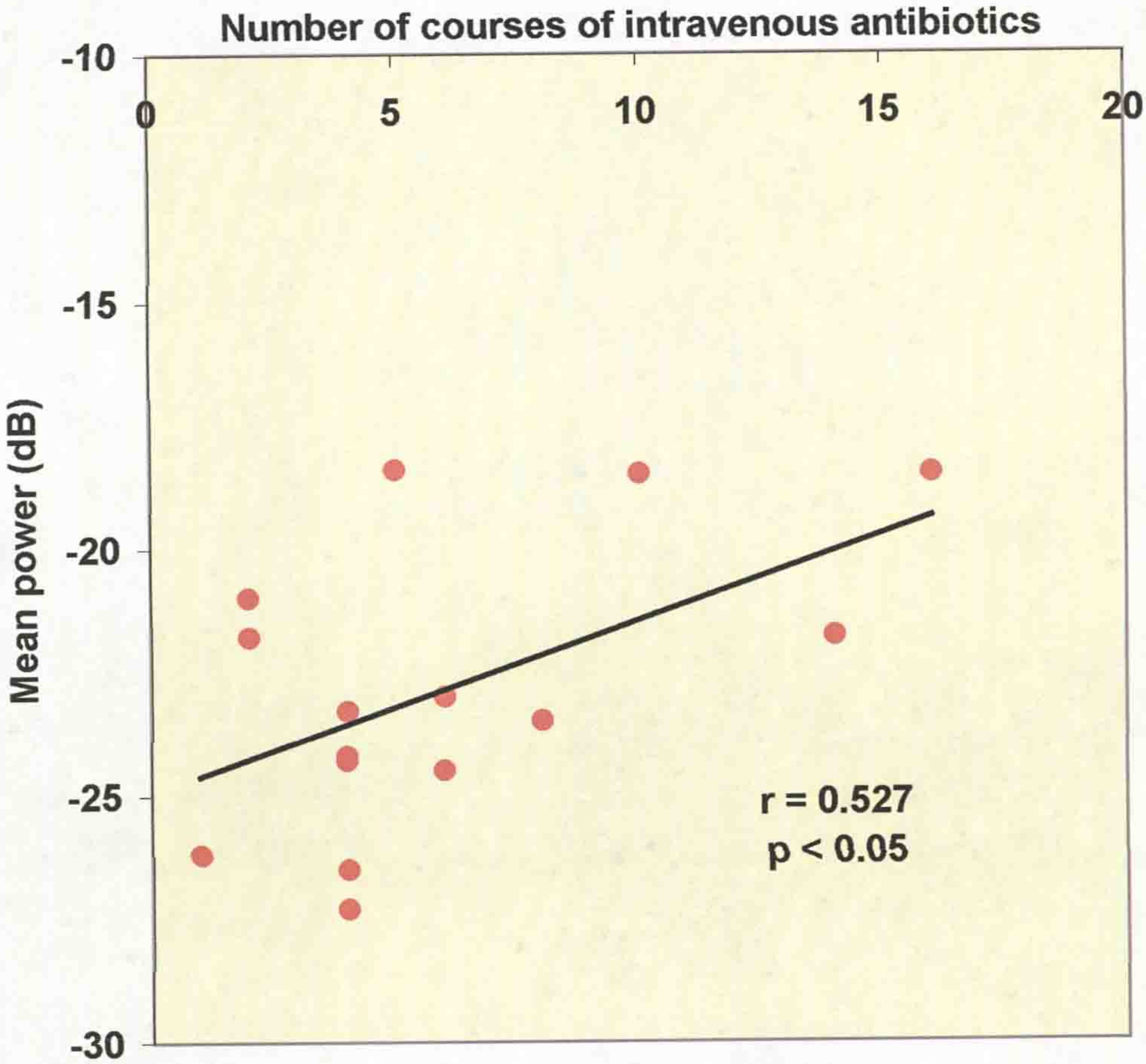


Fig 11: **Correlation between mean power and number of days on intravenous antibiotics over previous 2 years for 15 CF patients; information for number of days was unavailable for 1 patient**

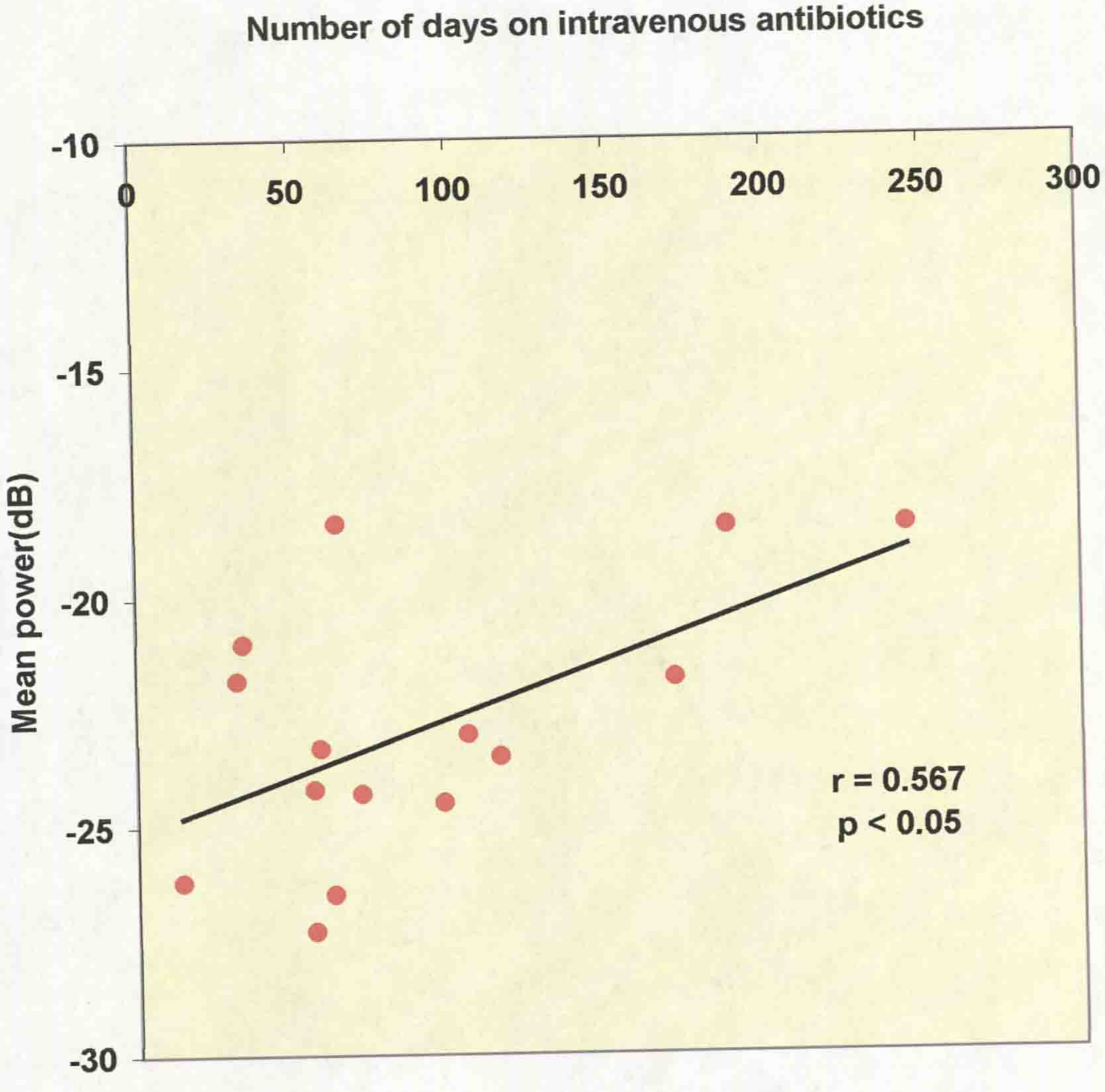


Fig 12: **Correlation between mean power and number of courses of intravenous antibiotics in diabetic patients (n = 7)**

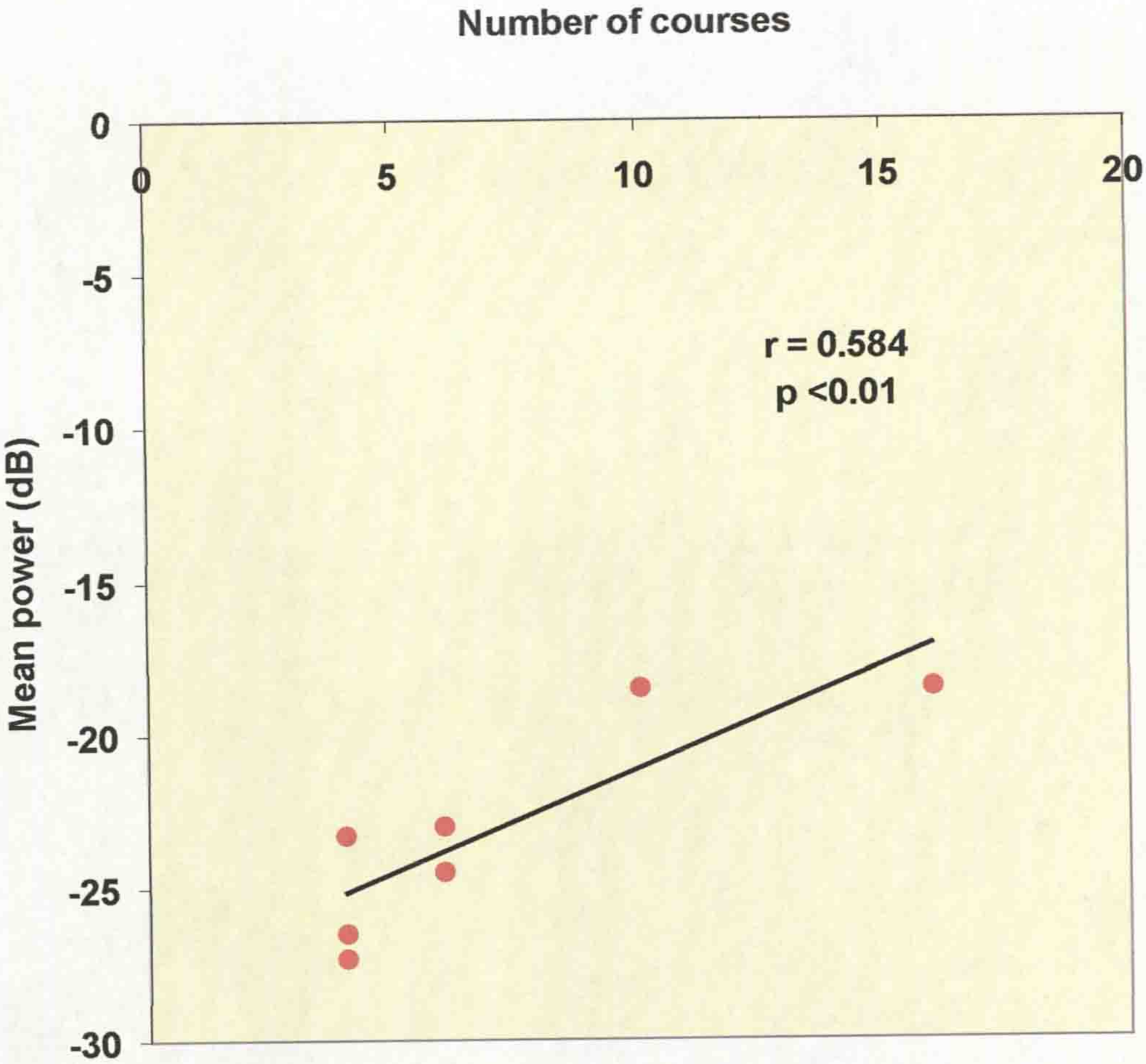
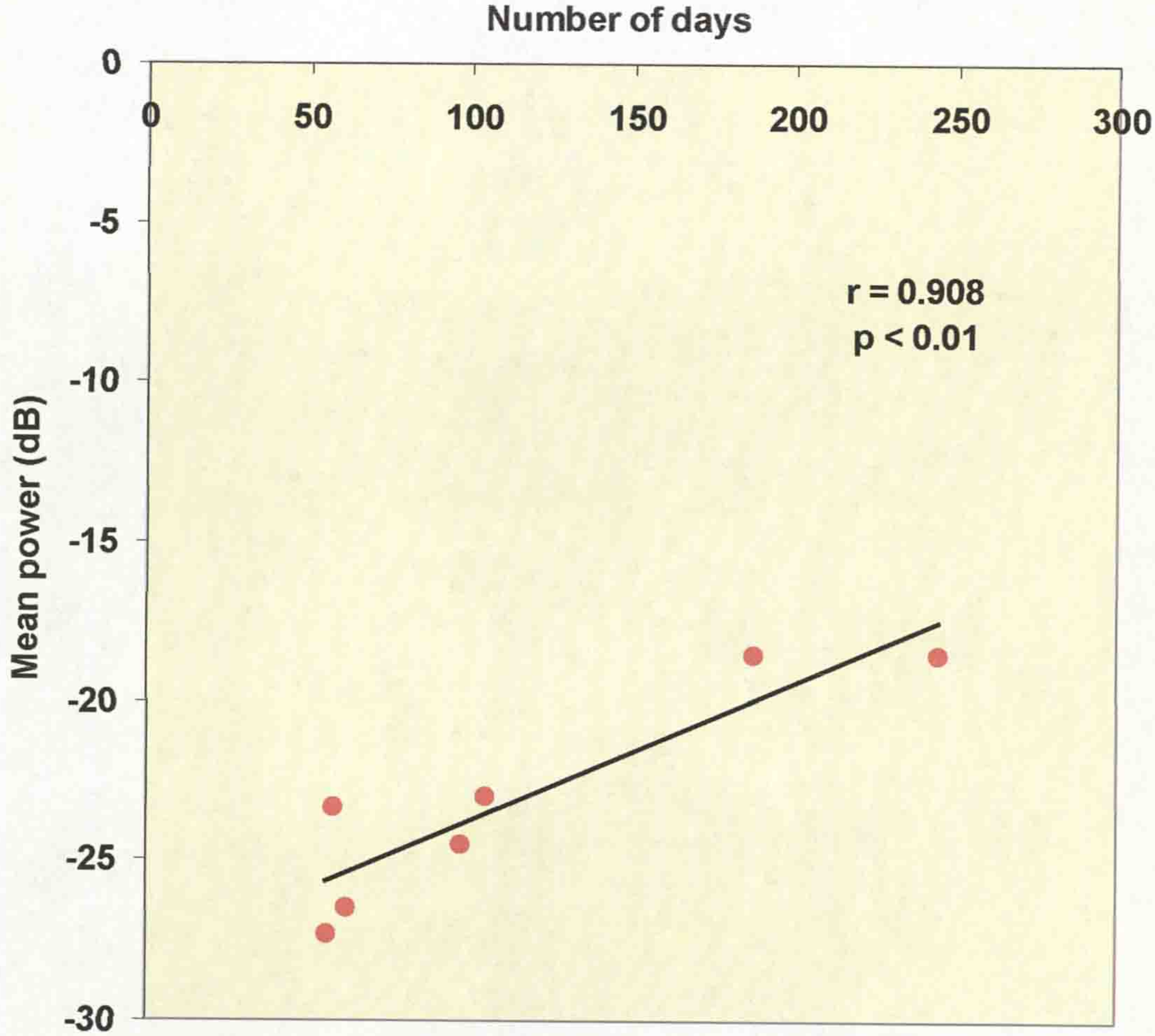


Fig 13: Correlation between mean power and number of days on intravenous antibiotics in diabetic patients (n = 7)



Summary of results

- Using the technique of spectral analysis, despite reasonable coefficients of variation for median frequency and mean power, there were no significant differences between the CF group as a whole and controls. However, there was a weakly significant difference in median frequency between diabetics and non-diabetics.

- In the whole CF group and the subgroup of diabetic patients, mean power correlated with the number of courses and number of days on intravenous antibiotics.

6:2 SPECTRAL ANALYSIS: DISCUSSION

Controls

Spectral analysis has demonstrated coefficients of variation for median frequency and mean power of 8.25% and 5.465% respectively. No comparable values are available in the literature.

In my controls, there was no correlation between mean power and median frequency with age, possibly reflecting the young age of this group (mean age 28.5 years). The range for median frequency (226.6Hz-515.6Hz) was comparable to the peak frequency in controls as reported by Yoshino et al (1990) (198Hz-500Hz) using a similar technique of bowel sound recording.

Patients

There was no correlation with age in this group.

There was no significant difference in mean power or median frequency between all CF patients and controls (-22.7dB versus –23.9dB, p=NS and 434.6Hz versus 455.3Hz, p=NS), non-constipated CF patients and controls (-22.9dB versus –23.9dB, p=NS and 435.8Hz versus 455.3Hz, p=NS), constipated CF patients and controls (-22.5dB versus –23.9dB, p=NS and 433Hz versus 455.3Hz, p=NS) or constipated and non-constipated CF patients (-22.5dB versus –22.9dB, p=NS and 433Hz versus 435.8Hz, p=NS). However, since none of the patients had a clinical diagnosis of DIOS (abdominal pain, right lower quadrant mass, abdominal X-ray with faecal material in the right colon), and since simple constipation may represent the mild end of this spectrum, this in not surprising. Nevertheless, a significant difference in median frequency was revealed when comparing diabetics and non-diabetics (481Hz versus 398.5Hz, p=0.018). As bowel sound in patients with obstruction have relatively higher frequencies (Yoshino et al, 1990) , it may be that the diabetic CF

patients were more prone to constipation, which could be due to neural dysfunction. Certainly, Battle et al (1980) showed that in comparison to controls, diabetics with mild or severe constipation had decreased motility (secondary to autonomic dysfunction as neostigmine and metoclopramide increased colonic spike and motor activity) which could encourage obstruction.

Only mean power for the whole CF group correlated with the number of courses and number of days spent on intravenous antibiotics over the previous 2 years. Therefore, if some patients have a greater number of pulmonary exacerbations of their disease, they may have more viscid mucus not just in their airways but also, for example, in their gastrointestinal tract. Coupled with dehydration, this could predispose towards constipation. Certainly Azmy and Ziervogel (1983) reported that in children with CF, meconium ileus equivalent could be precipitated by reduction or stopping pancreatic enzyme supplements, dehydration or intercurrent chest infections.

In addition, diabetic patients also demonstrated a correlation between mean power and requirement for intravenous antibiotics.

CHAPTER 7: GASTROINTESTINAL SYSTEM/ BOWEL SOUNDS (II)

7:1 COOLEDIT: RESULTS

7:2 COOLEDIT: DISCUSSION

7:1 COOLEDIT: RESULTS

The number of bowel sounds per minute above 20% amplitude was recorded for all subjects.

Control subjects

16 controls took part, 1 male and 15 females. Mean age was 28.8 years, range 22 to 35 years. The mean number of bowel sounds per minute (BS/min) was 4.54, range 0.59 to 14.11. There was no correlation with age (r=0.169, p=NS).

Repeatability study

The bowel sounds of two control subjects were recorded in the supine position 3-4 hours after a meal at the same time on 2 successive days, the microphone was taped to the left lower abdomen and the coefficient of variation calculated as 82.3% (Table 1).

Table 1: Repeatability study in 2 control subjects

SUBJECT	BS/min (DAY 1)	BS/min (DAY 2)	BS/min (DAY 3)
1	7.04	2.62	1.99
2	0.63	10.08	4.5

Patients

There were 17 patients in total, 6 male and 11 female. The mean age was 23.4 years, range 19 to 42 years. Mean number of BS/min was 10.35, range 0.69 to 53.04. as for the controls, there was no correlation with age (r=-0.042, p=NS). Table 2 shows the mean values for markers of disease severity.

Patients with constipation

7 of these patients had constipation at the time of recording, 1 male and 6 female. Mean age was 21.9 years (range 19 to 29 years) and mean BS/min was 12.78 (range 2.42 to 29.47). Markers of disease severity are indicated in Table 3.
When constipation had been treated (medically), mean BS/min was 5.34 (range 0.09 to 18.24).

Patients without constipation

There were 10 such patients, 5 male and 5 female, with a mean age of 24.7 years (range 19 to 42 years) and mean BS/min of 8.65 (range 0.69 to 53.04). Values for markers of disease severity are given in Table 4.

Table 2: Mean values for markers of disease severity in the patient group

PARAMETER	MEAN VALUE (RANGE)
Age (years)	23.4 (19-42)
FEV1 (% predicted)	64.1 (24-88)
FVC (% predicted)	78.3 (45-106)
O2 saturations (%)	97.3 (95-100)
BMI (kg/m^2)	20.2 (13.9-23.7)
Fasting glucose (mmol/l)	8.5 (4-22.4)
HbA1c (%)	6.6 (5.5-10.1)
Vitamin E (umol/l)	13.3 (4-27)
No of courses of iv antibiotics over previous 2 yrs	6.1 (1-16)
No of days on iv antibiotics over previous 2 yrs	93.4 (14-245)

Table 3: Mean values for markers of disease severity in constipated CF patients (n = 7)

PARAMETER	MEAN VALUE (RANGE)
Age (years)	21.9 (19-29)
FEV1 (% predicted)	79.2 (64-88)
FVC (% predicted)	86.2 (83-92)
O2 saturations (%)	98.3 (97-100)
BMI (kg/m^2)	20.9 (18.1-23.7)
Fasting glucose (mmol/l)	8.7 (4.3-16.5)
HbA1c (%)	6.2 (5.5-6.9)
Vitamin E (umol/l)	13.4 (4-23)
No of courses of iv antibiotics over previous 2 yrs	7.3 (4-14)
No of days on iv antibiotics over previous 2 yrs	107 (56-188)

Table 4: Mean values for markers of disease severity in non-constipated patients (n = 10)

PARAMETER	MEAN VALUE (RANGE)
Age (years)	24.7 (19-42)
FEV1 (% predicted)	54.1 (24-82)
FVC (% predicted)	73 (45-106)
O2 saturations (%)	96.7 (95-98)
BMI (kg/m^2)	19.6 (13.9-23)
Fasting glucose (mmol/l)	8.4 (4-22.4)
HbA1c (%)	6.9 (5.7-10.1)
Vitamin E (umol/l)	13.3 (4-27)
No of courses of iv antibiotics over previous 2 yrs	5.2 (1-16)
No of days on iv antibiotics over previous 2 yrs	82.9 (14-245)

Comparison between all CF patients and controls

There was no significant difference between these groups for BS/min (CF mean 10.35 versus controls 4.54, p=NS) (Fig 1) even when subjected to a logarithmic conversion (CF mean 0.78 versus controls 0.48, p=NS) (Fig 2).

Comparison between male and female patients

This revealed no significant differences either for BS/min (males 8.42, females 11.41, p=NS) (Fig 3) or markers of disease severity (Table 5).

Comparison between diabetic and non-diabetic patients

As before, those patients on regular insulin therapy were classed as diabetic. 7 diabetic and 10 non-diabetic patients participated. Again, there were no differences for BS/min (diabetics 11.13, non-diabetics 9.81, p=NS) (Fig 4) or markers of disease severity (Table 6).

Comparison between non-constipated CF patients and controls

The difference in BS/min was not significant (CF mean 8.65 versus controls 4.54, p=NS) (Fig 5) even after logarithmic transformation (CF mean 0.63 versus controls 0.48, p=NS) (Fig 6).

Comparison between constipated CF patients and controls

Mean values for BS/min were: CF patients 12.78, controls 4.54 (p=NS) (Fig 7). However, comparison of the logarithmic values revealed a significant difference: CF patients 0.99, controls 0.48, p=0.01 (Fig 8).

Comparison between constipated and non-constipated CF patients

Again, no significant difference was found in BS/min either for absolute values (constipated patients mean 12.78, non-constipated mean 8.65, p=NS) (Fig 9) or logarithmic values (0.99 versus 0.63, p=NS) (Fig 10). Only FEV1 (% predicted) differed significantly between the groups (Table 7).

Comparison between patients when constipated and following treatment

Mean BS/min did not differ between these two states (constipated 12.78, treated 5.34, p=NS, Fig 11): after log transformation of the number of bowel sounds per minute, constipated 0.99, treated 0.11, p=NS, Fig 12).

Correlation with markers of disease severity

There were no significant correlations.

Fig 1: Comparison of bowel sounds/min between controls (n=16) and all CF patients (n=17)

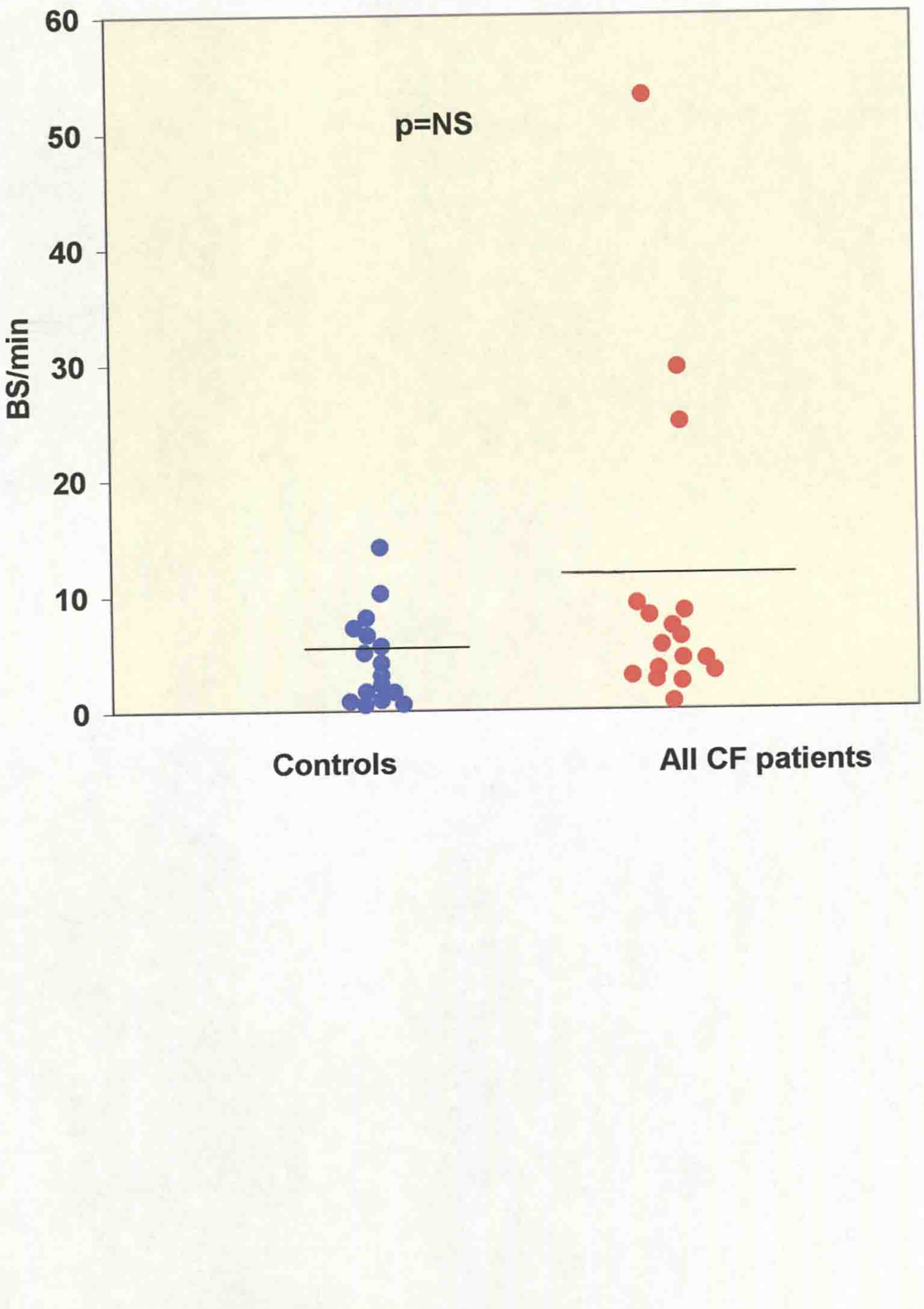


Fig 2: **Comparison of bowel sounds/min between controls (n=16) and all CF patients (n=17) after log transformation**

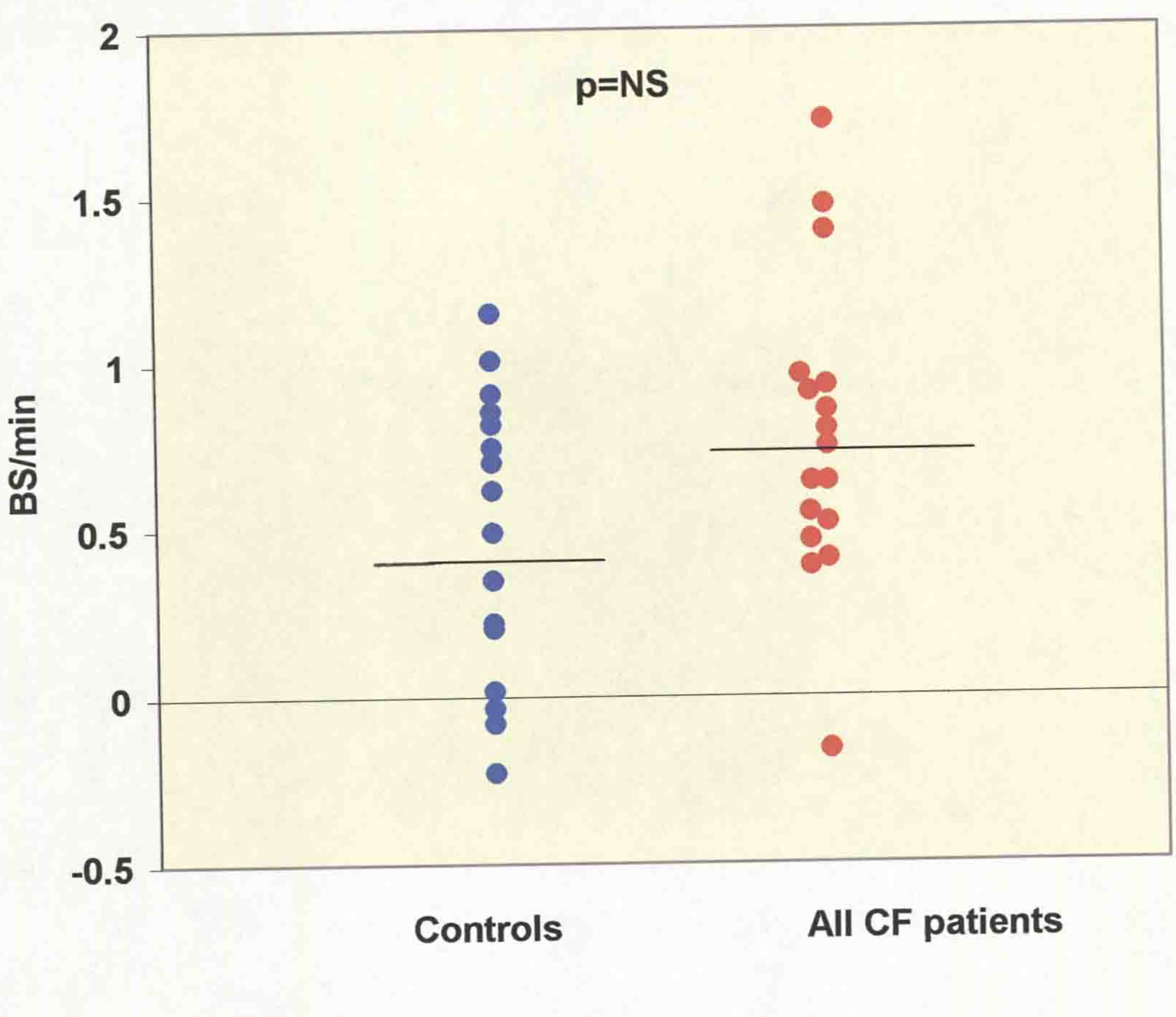


Fig 3: **Comparison of bowel sounds/min between male (n=6) female patients (n=11)**

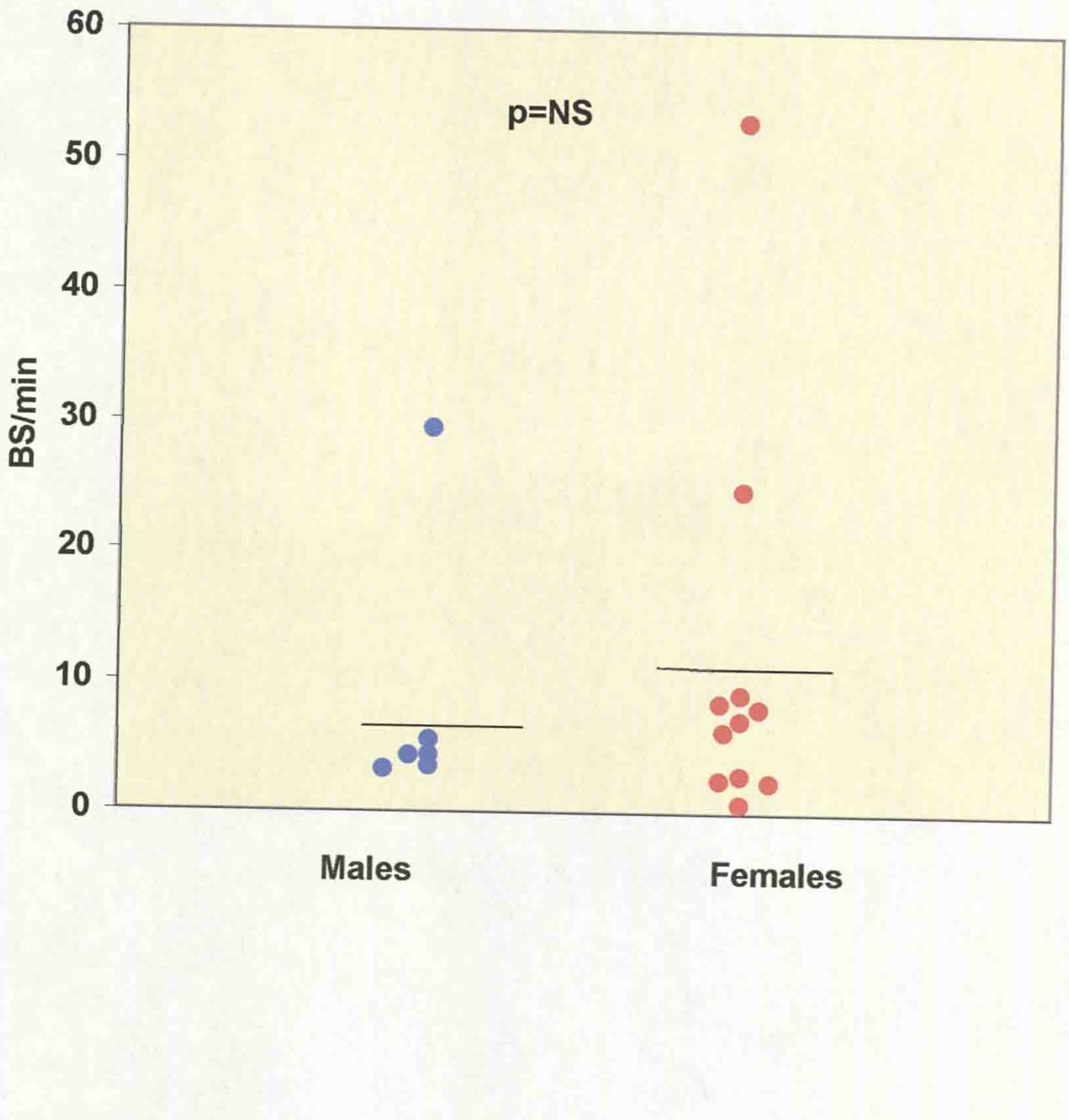


Table 5: Comparison between male (n = 6) and female patients (n= 11)

PARAMETER	MEAN VALUE FOR MALES (RANGE)	MEAN VALUE FOR FEMALES (RANGE)	P VALUE
BS/min	8.42 (3.28-29.47)	11.41 (0.69-53.04)	0.640
Age (yrs)	24.2 (19-29)	23.1 (19-42)	0.684
FEV1 (% predicted)	50.5 (24-79)	69.1 (40-88)	0.268
FVC (% predicted)	73 (45-106)	80.2 (49-101)	0.617
O2 saturations (%)	96 (95-98)	97.8 (96-100)	0.080
BMI (kg/m^2)	19.9 (13.9-23.7)	20.4 (18.1-22.6)	0.793
Fasting glucose (mmol/l)	10.8 (4.3-22.4)	7.3 (4-16.5)	0.365
HbA1c (%)	7.0 (5.8-10.1)	6.4 (5.5-7.7)	0.558
Vitamin E (umol/l)	13.4 (6-23)	13.3 (4-27)	0.973
No of courses of iv antibiotics over previous 2 yrs	7.2 (2-16)	5.6 (1-14)	0.606
No of days of iv antibiotics over previous 2 yrs	120.8 (32-245)	81 (14-171)	0.409

Fig 4: Comparison of bowel sounds/min between diabetic (n=7) non-diabetic CF patients (n=10)

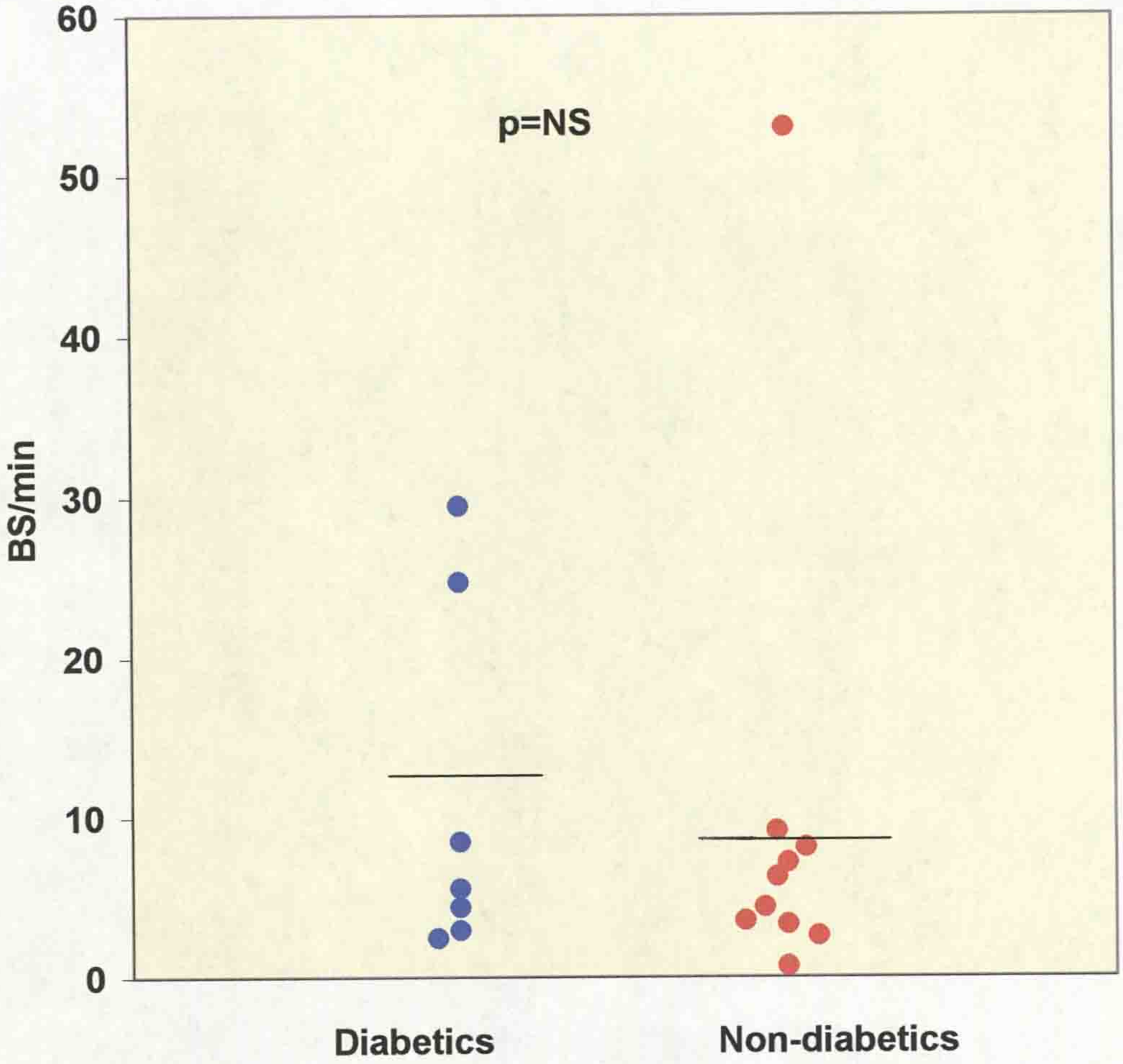


Table 6: Comparison between diabetic (n = 7) and non-diabetic patients (n=10)

PARAMETER	MEAN VALUE (DIABETICS) (RANGE)	MEAN VALUE (NON-DIABETICS) (RANGE)	P VALUE
BS/min	11.14 (2.42-29.47)	9.81 (0.69-53.04)	0.839
Age (yrs)	25.6 (19-42)	21.8 (19-27)	0.270
FEV1 (% predicted)	63.5 (24-88)	64.6 (31-82)	0.934
FVC (% predicted)	86 (45-106)	73.1 (49-85)	0.227
O2 saturations (%)	97.5 (96-100)	97.2 (95-99)	0.727
BMI (kg/m^2)	19.6 (13.9-23.7)	20.8 (17.8-23)	0.407
Vitamin E (umol/l)	12 (4-23)	14.4 (4-27)	0.569
No of courses of iv antibiotics over previous 2 yrs	7.1 (4-16)	5.3 (1-14)	0.420
No of days of iv antibiotics over previous 2 yrs	115.8 (56-245)	76 (14-171)	0.249

Fig 5: **Comparison of bowel sounds/min between controls (n=16) and non-constipated CF patients (n=10)**

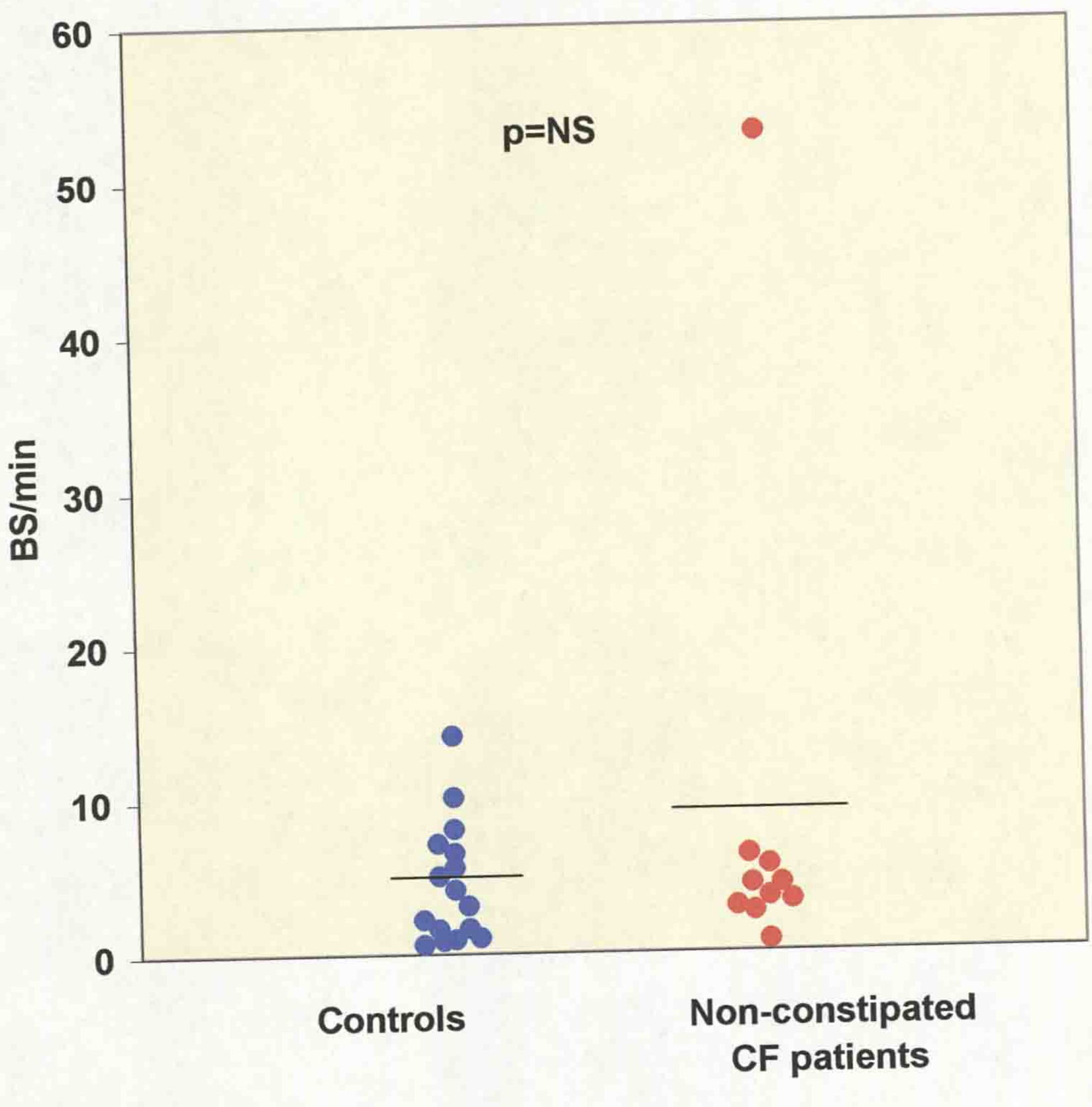


Fig 6: **Comparison of bowel sounds/min between controls (n=16) and non-constipated CF patients (n=10) after log transformation**

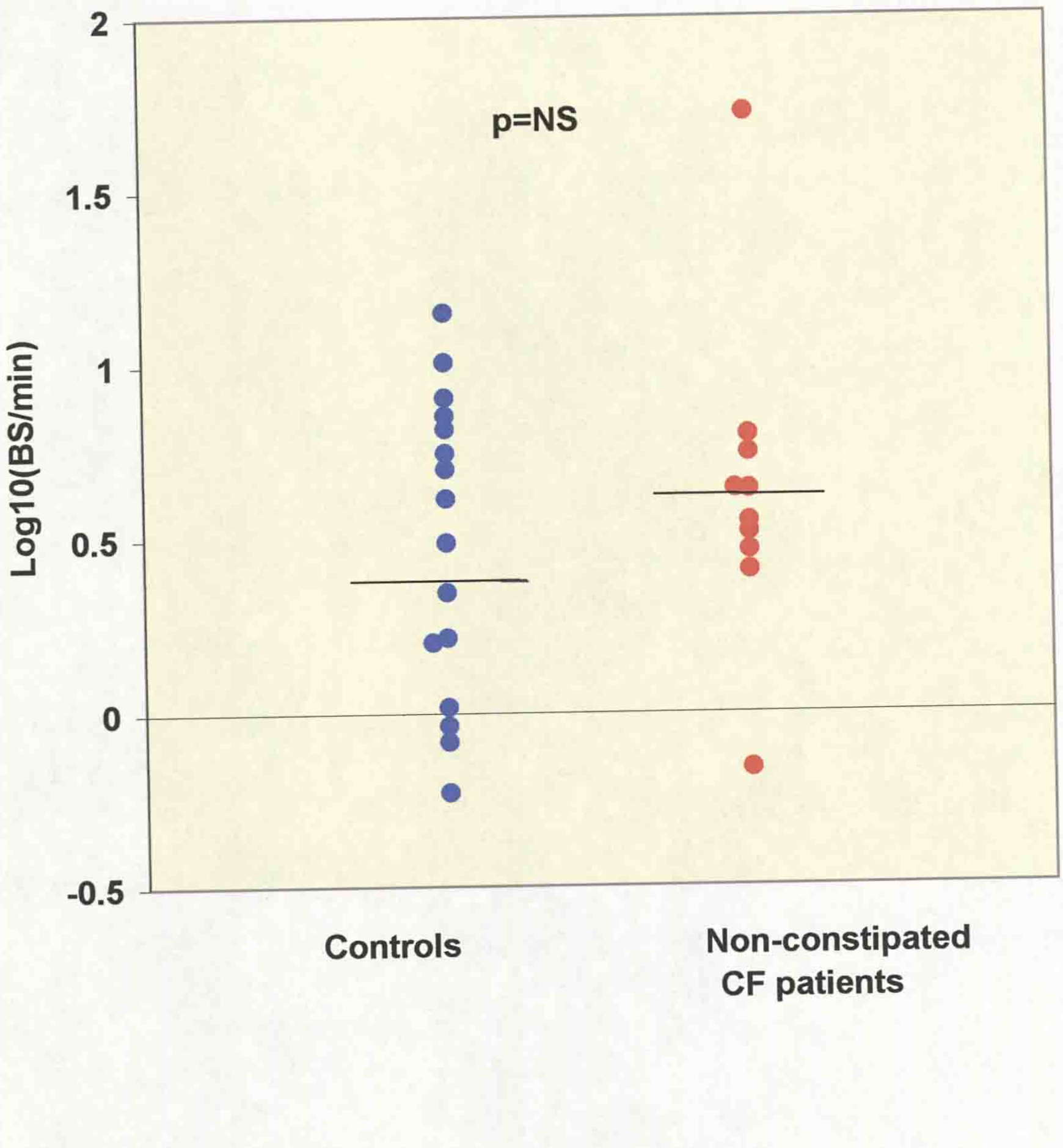


Fig 7: Comparison of bowel sounds/min between controls (n=16) and constipated CF patients (n=7)

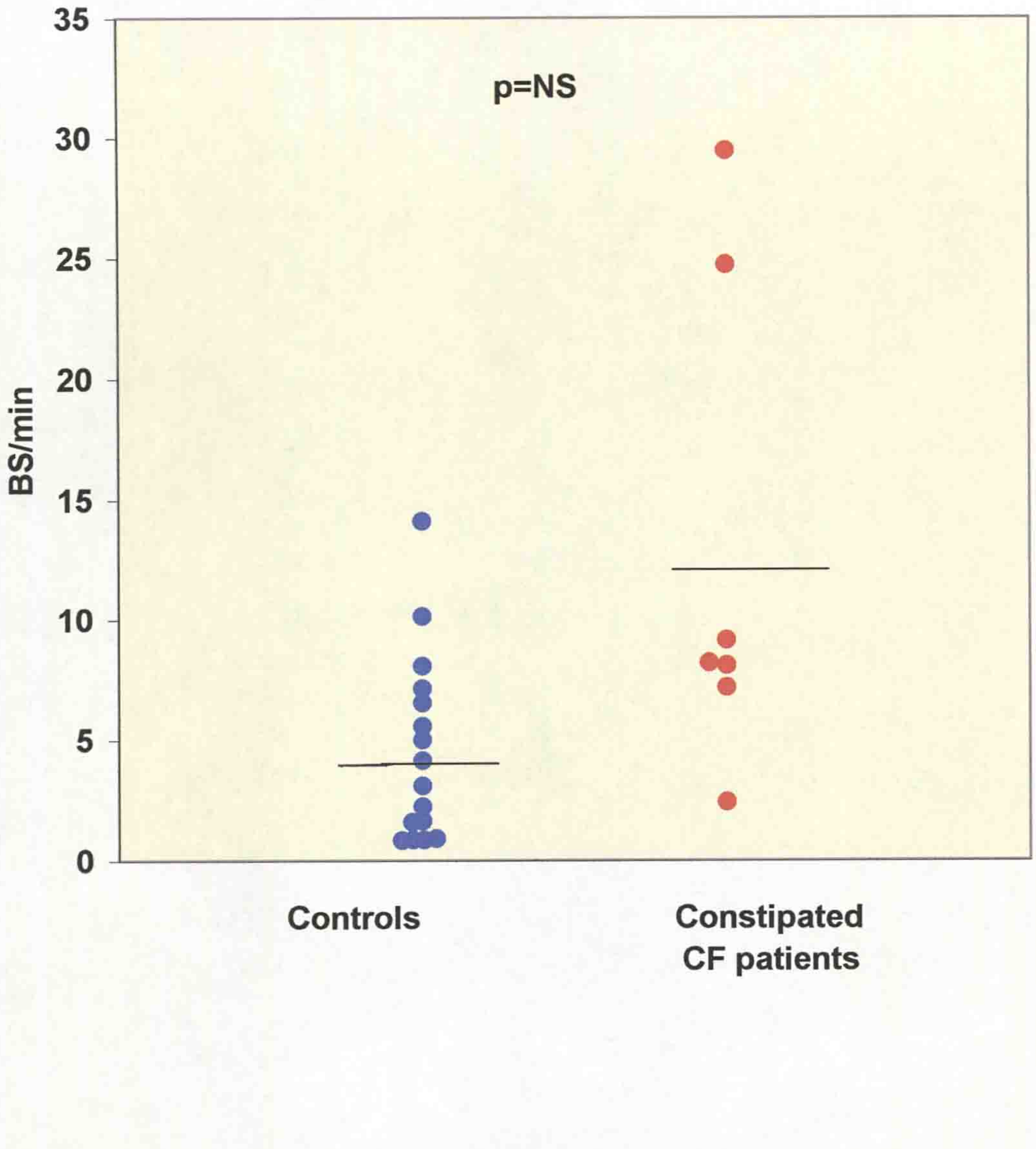


Fig 8: **Comparison of bowel sounds/min in controls (n=16) and constipated CF patients (n=7) after log transformation**

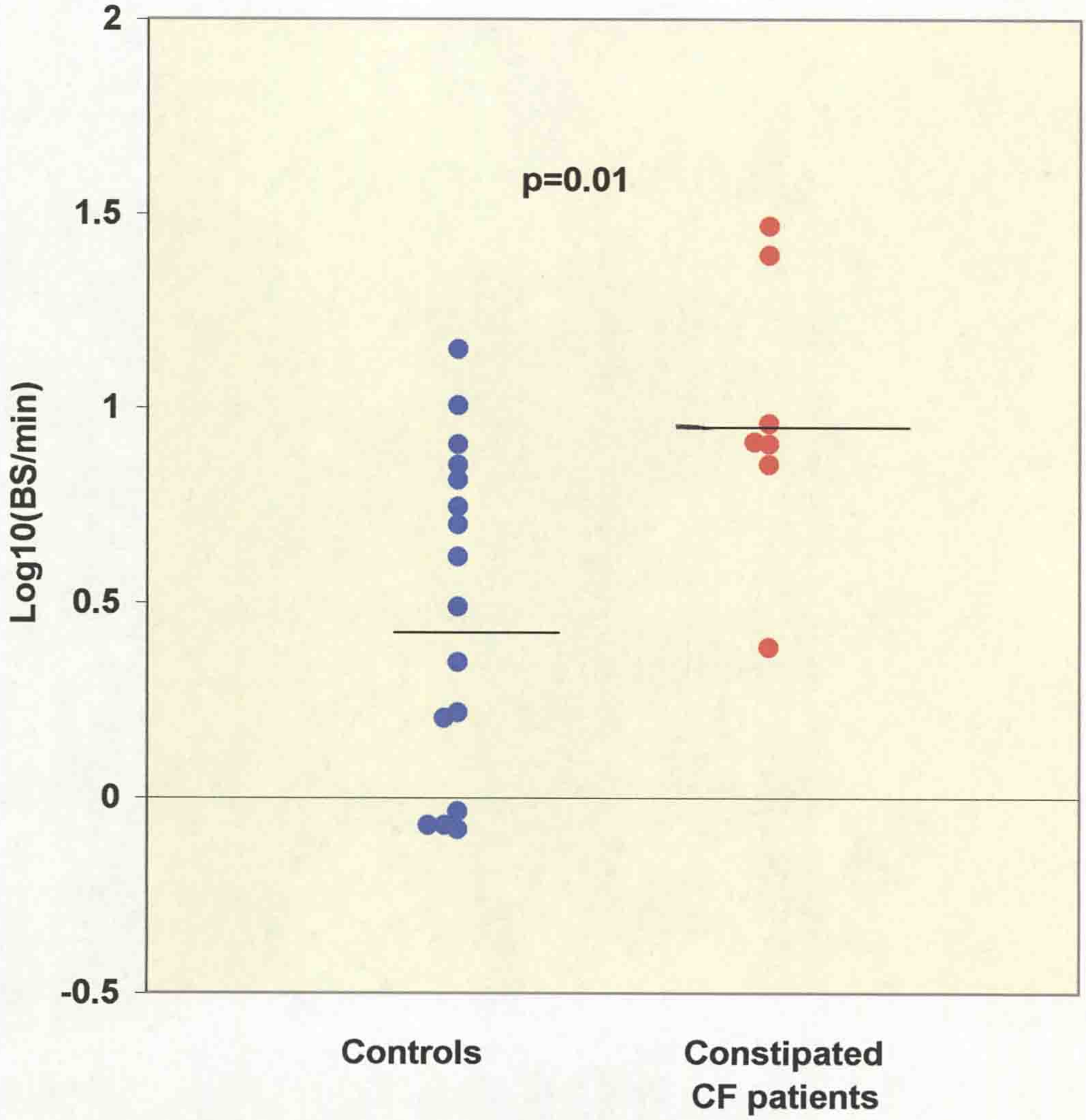


Fig 9: Comparison of bowel sounds/min between constipated (n=7) non-constipated CF patients (n=10)

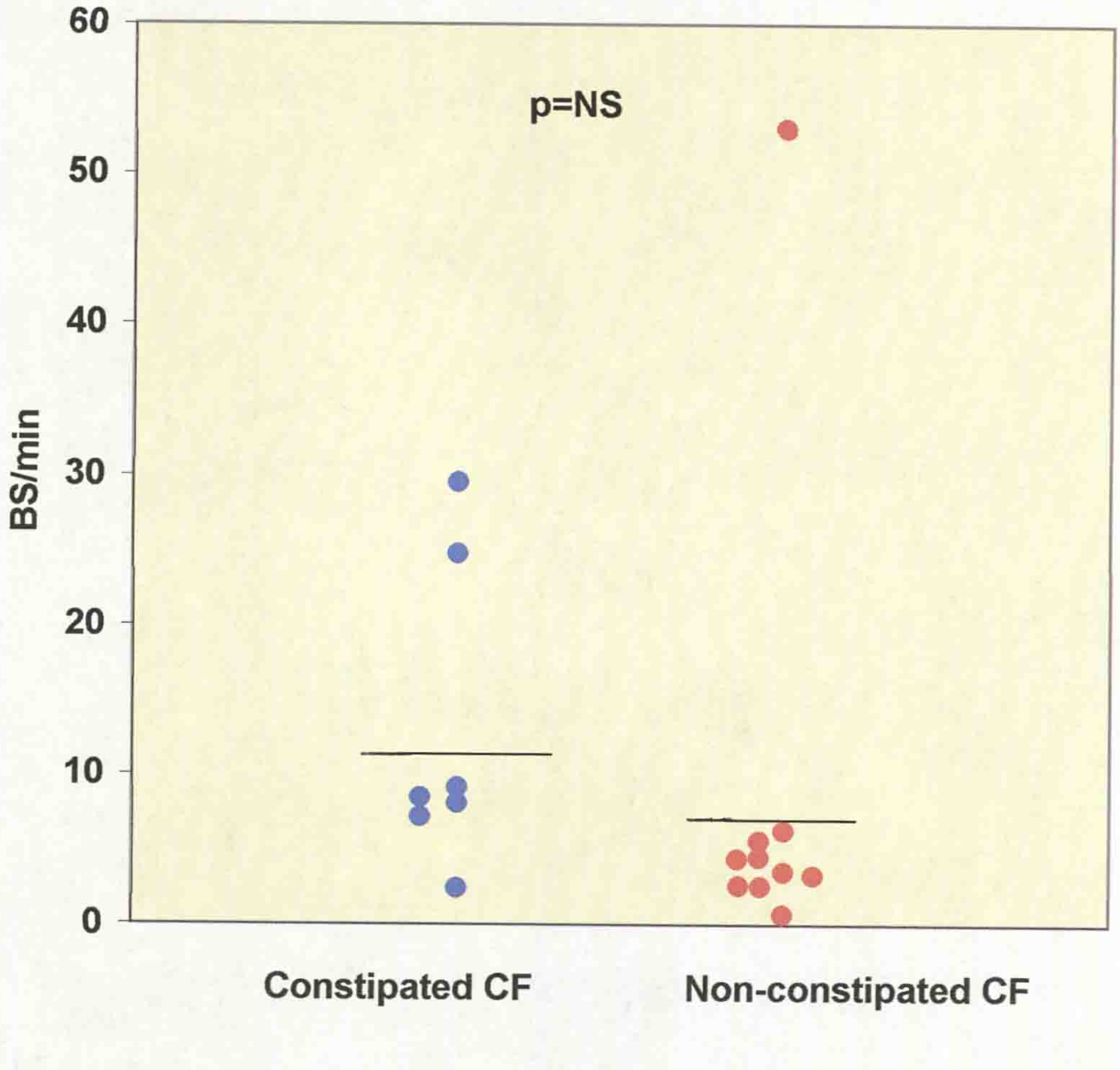


Fig 10: **Comparison of bowel sounds/min between constipated (n=7) and non-constipated CF (n=10) patients after log transformation**

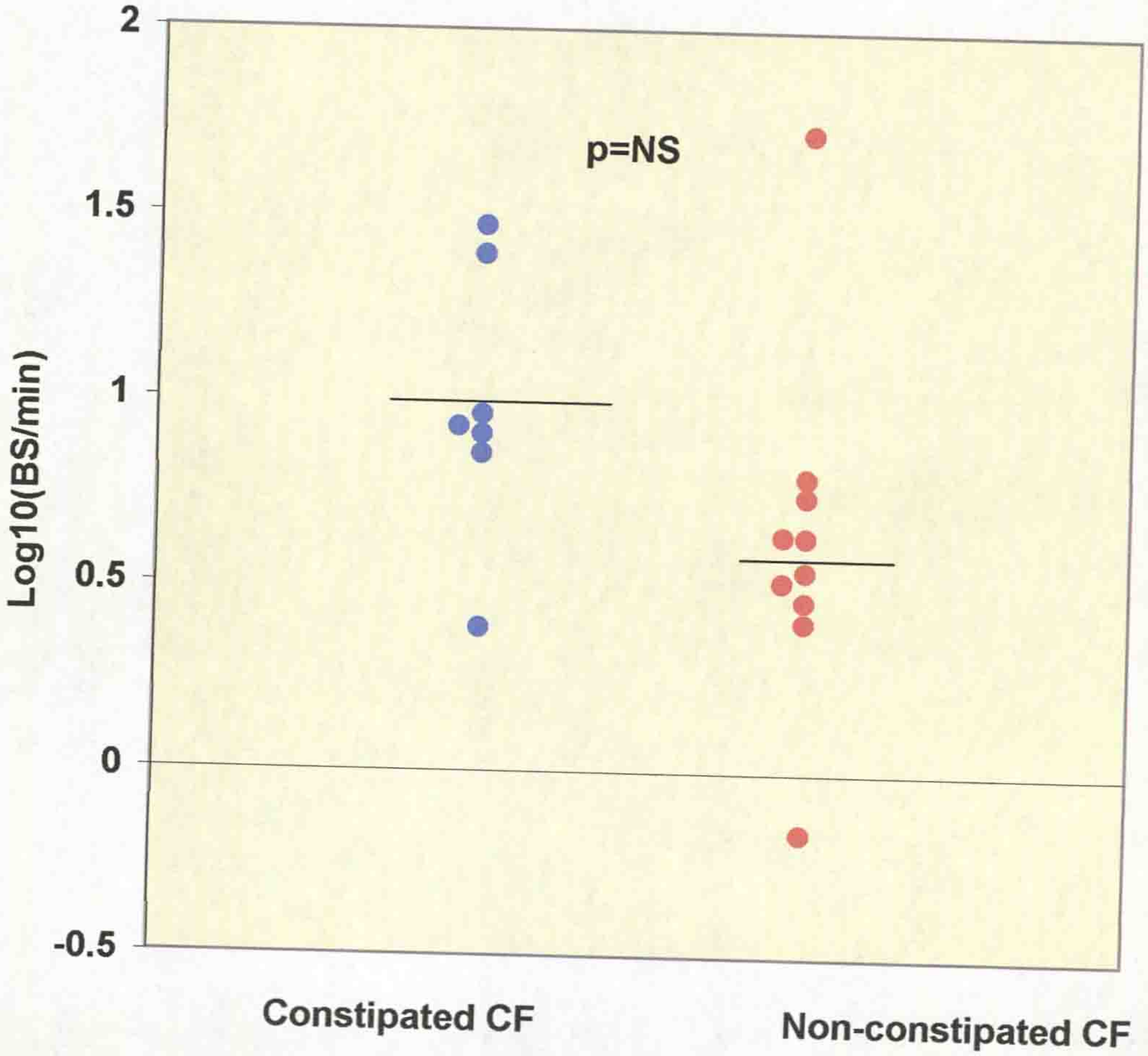


Table 7: Comparison of markers of disease severity between constipated (n=7) and non-constipated CF patients (n=10)

PARAMETER	MEAN VALUE (CONSTIPATED) (RANGE)	MEAN VALUE (NON-CONSTIPATED) (RANGE)	P VALUE
Age (years)	21.9 (19-29)	24.7 (19-42)	0.321
FEV1 (% predicted)	79.2 (64-88)	54.1 (24-82)	0.007
FVC (% predicted)	86.2 (83-92)	73 (45-106)	0.095
O2 saturations (%)	98.3 (97-100)	96.7 (95-98)	0.01
BMI (kg/m^2)	20.9 (18.1-23.7)	19.6 (13.9-23)	0.296
Fasting glucose (mmol/l)	8.7 (4.3-16.5)	8.4 (4-22.4)	0.916
HbA1c (%)	6.2 (5.5-6.9)	6.9 (5.7-10.1)	0.163
Vitamin E (umol/l)	13.4 (4-23)	13.3 (4-27)	0.974
No of courses of iv antibiotics over previous 2 yrs	7.3 (4-14)	5.2 (1-16)	0.335
No of days on iv antibiotics over previous 2 yrs	107 (56-188)	82.9 (14-245)	0.452

Fig 11: Comparison of bowel sounds/min between CF patients when constipated (n=7) and following treatment (n=4)

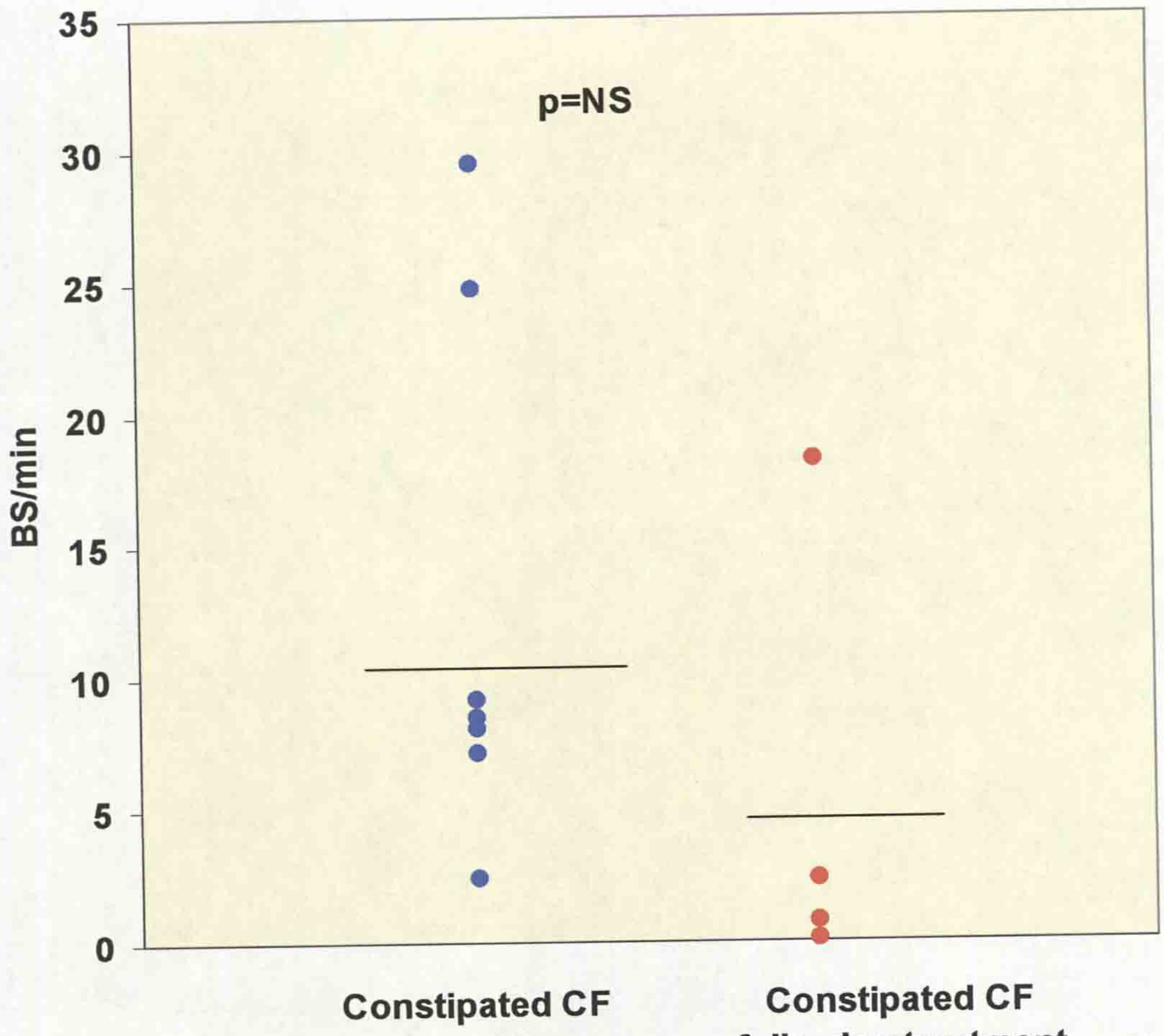
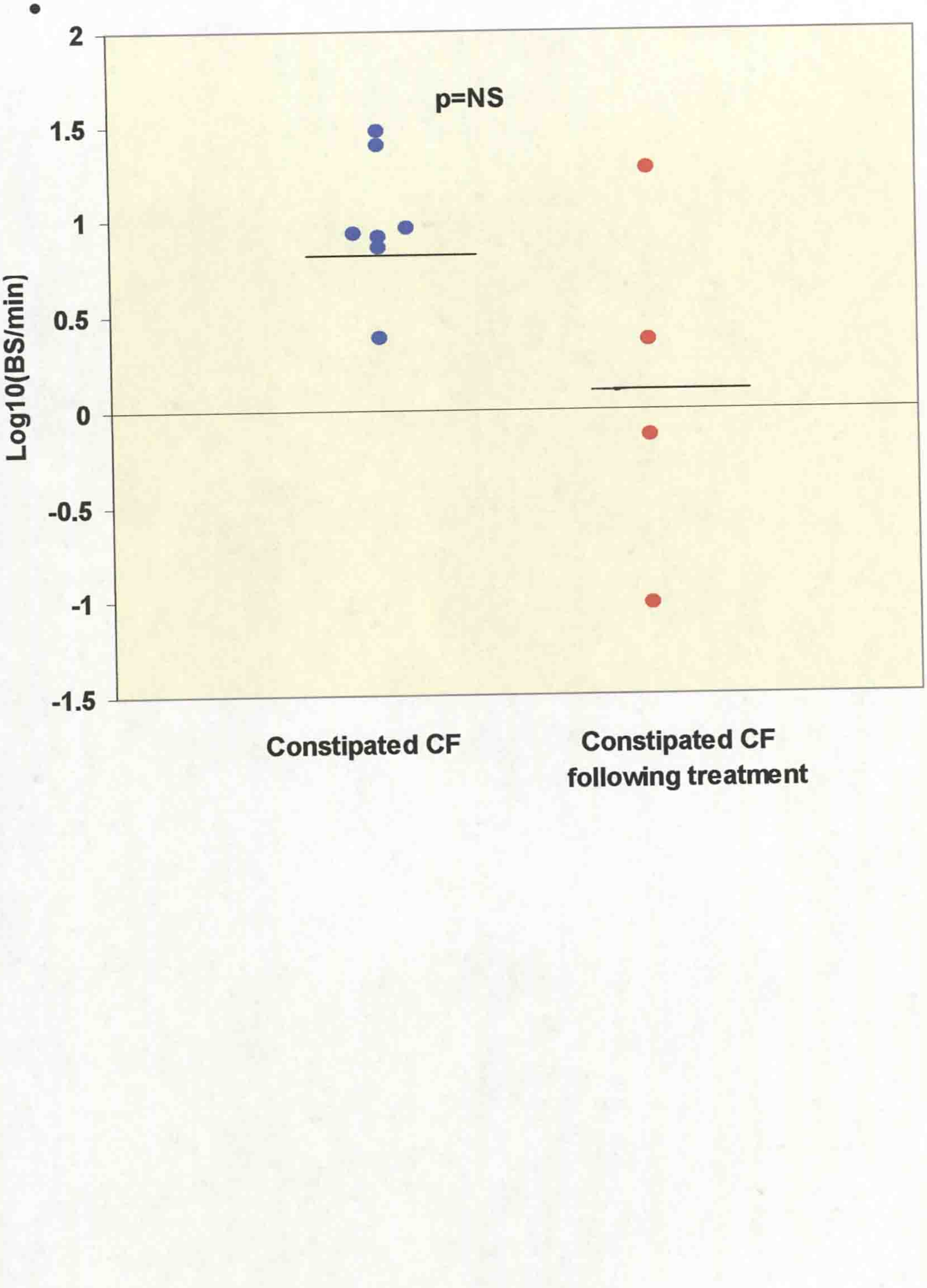


Fig 12: **Comparison of bowel sounds/min between CF patients when constipated (n=7) and following treatment (n=4) after log transformation**



Summary of results

- When using 'CoolEdit', log_{10}BS/min differed significantly between constipated CF patients and controls only.
- There were no significant correlations between bowel sound parameters and markers of disease severity in any of the groups.

7:2 COOLEDIT: DISCUSSION

The simplest parameter which can be measured using CoolEdit is the number of bowel sounds per minute. Previous work in the literature has suggested that measurement of the number of bowel sounds is a suitable way of distinguishing obstructed patients from normal controls. For example, Arnbjornsson (1986) showed that in comparison with controls, patients with mechanical small bowel obstruction had a higher motility index as well as 3 to 10 regular high amplitude sounds preceded and followed by at least one minute of absent motor activity. Sugrue and Redfern (1994) reported that the number of bowel sounds in obstructed patients was decreased in comparison to controls (number of sounds per second in controls 0,6 versus patients 0.4, $p<0.05$). Other parameters which could potentially be measured include sound length, sound to silence ratio, the interval between successive sounds and the mean amplitude (loudness). However, analysis of these requires the use of much more complex programmes than those offered by CoolEdit.

Controls

There are no previous reports in the literature regarding the use of the CoolEdit programme for the analysis of bowel sounds. For my control group, there was a wide range of BS/min (0.59-14.11) and again no correlation with age.

The repeatability study demonstrated a poor coefficient of variation. This may be because bowel sounds themselves are influenced by a number of variables such as ambient noise, the nature of a meal, intraluminal pressure, the proportion of gas and liquid, flow rate and the nature of the bowel contents such as their viscosity. The size of the abdominal cavity and the presence of muscle or fat can also affect the recordings (Arnbjornsson, 1986). Some of these variables were difficult to control for. However, all subjects had bowel sounds recorded 3 to 4 hours after a meal, rather than immediately afterwards as the duration of the post-prandial motility pattern, commonly 3 to 4 hours, depends heavily on the caloric content of the meal. In addition, all subjects were examined in quiet surroundings to minimise pollution from unwanted extraneous noise, although

during bowel sound analysis it was fairly straightforward to distinguish a bowel sound from these other noises as CoolEdit possesses an audio facility. Other variables were difficult to standardise.

Patients

There was an even wider range for BS/min in this group (0.69-53.04). No correlation with age was found.

Comparisons of mean BS/min between the various groups revealed no significant differences between all CF patients and controls, males and females, diabetics and non-diabetics, non-constipated patients and controls, and constipated and non-constipated CF patients. However, after logarithmic transformation, a significant difference in BS/min was observed between controls and constipated patients (0.48 versus 0.99, $p<0.01$), indicating that bowel sounds were more frequent in the latter. Thus, even though bowel sound analysis using CoolEdit has a poor reproducibility, it has demonstrated significant differences in motility. Furthermore, when the constipated patients were treated medically, BS/min were decreased (12.78 to 5.34) although the difference was not statistically significant.

There was no correlation between BS/min for any of the patient groups and markers of disease severity.

CHAPTER 8: URINARY SYSTEM

8:1 URINARY SYSTEM: RESULTS

8:2 URINARY SYSTEM: DISCUSSION

8:1 URINARY SYSTEM: RESULTS

No control subjects were included in the study as the Liverpool nomogram was used to define which centile a patient was on as determined by the maximum and average urine flow rates corresponding to the particular voided volume.

Patients

5 patients participated in the study, all female. Their mean age was 21.8 years, range 20 to 24 years.

Table 1 shows the mean values for each of the parameters of urine flow. Full uroflowmetry data was available for 4 patients. Post voiding ultrasound examination of the urinary tract showed no evidence of mechanical obstruction in any patient.

Table 2 shows the centiles corresponding to each patient using the Liverpool nomogram for women (Haylen et al, 1989). A subsequent study by Haylen et al (1990) reported that in women the 10^{th} centile for both flow rates described a useful lower limit of normality.

Markers of disease severity are indicated in Table 3.

Correlations with markers of disease severity

There were no correlations with FEV1, FVC or body mass index. However, residual volume correlated with vitamin E levels ($r=0.998$, $p<0.05$) (Fig 1) and number of courses and days on intravenous antibiotics over the previous 2 years ($r=0.881$, $p<0.05$ and $r=0.928$, $p<0.05$ respectively) (Figs 2 and 3). There was a negative correlation between maximum flow rate and fasting glucose concentrations ($r=-0.974$, $p<0.05$) (Fig 4). None of these patients was known to be diabetic.

Table 1: Mean values and ranges for parameters of urine flow

PARAMETER	MEAN VALUE (RANGE)
Residual volume of urine (mls)	35.4 (9-77)
Maximum flow rate (mls/s)	15.4 (7-19.1)
Average flow rate (mls/s)	8.2 (2.5-13)
Voided volume (mls)	187.3 (31-282)

Table 2: Patients' centiles for maximum and average urine flow rates

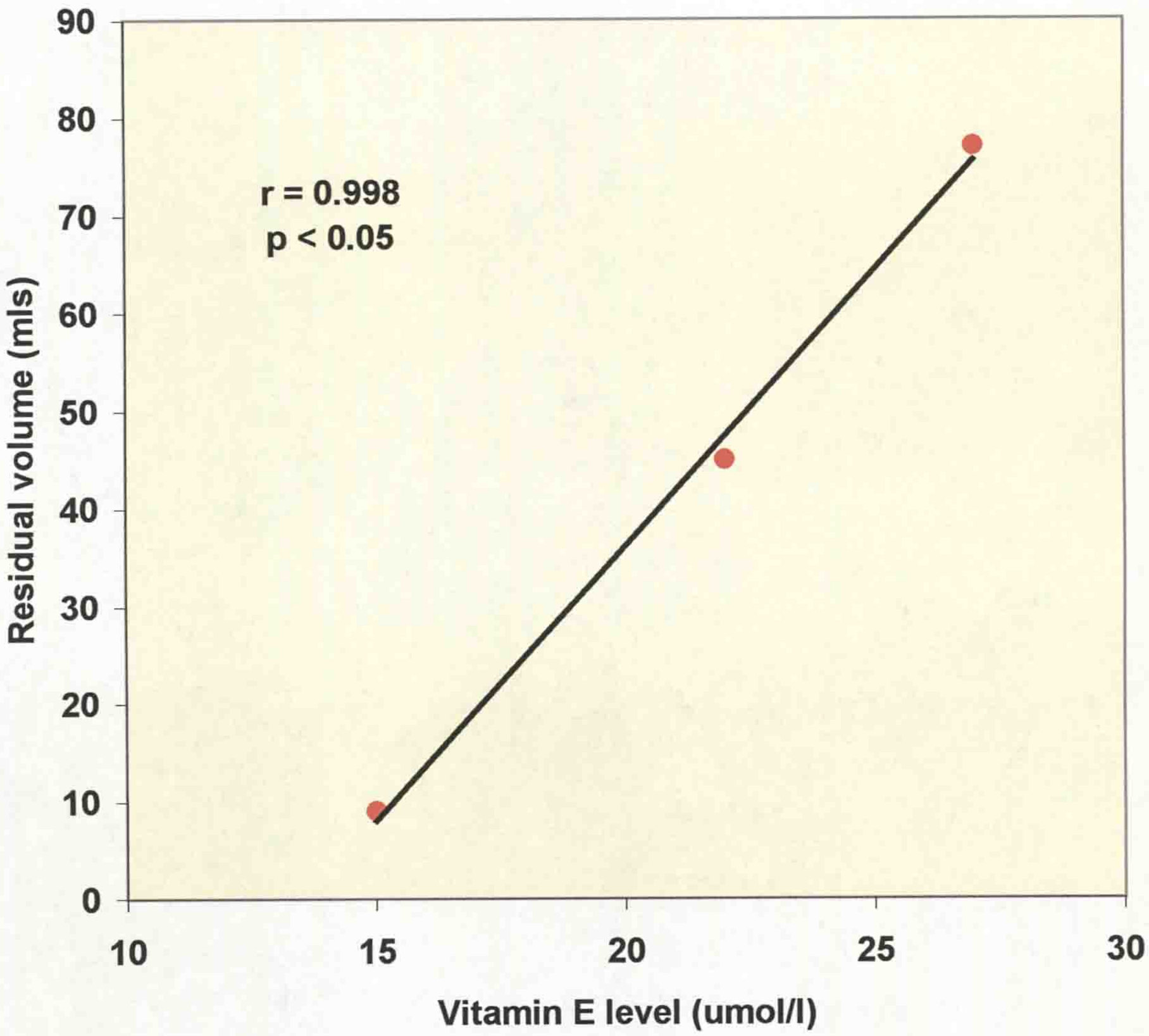
Patient	Voided volume (mls)	Max flow rate (mls/sec)	Centile	Average flow rate (mls/sec)	Centile
2	282	18	10	9.1	<10
3	31	7	25	2.5	25
4	174	19.1	25	13	50
5	262	17.6	10	8	<10

Complete data was not available for Patient 1

Table 3: Mean values and ranges for markers of disease severity

PARAMETER	MEAN VALUE (RANGE)
Age (yrs)	21.8 (20-24)
FEV1 (% predicted)	48.4 (27-63)
FVC (% predicted)	62.2 (49-77)
O2 saturations (%)	97 (96-98)
BMI (kg/m^2)	18.9 (14.9-22.6)
Fasting glucose (mmol/l)	5.0 (4-6.9)
HbA1c (%)	6.4 (5.7-7)
Vitamin E (umol/l)	21.3 (15-27)
No of courses of iv antibiotics over previous 2 yrs	5.4 (4-8)
No of days on iv antibiotics over previous 2 yrs	81.2 (56-127)

Fig 1: Correlation between residual volume and vitamin E levels in female CF patients



Vitamin E levels were only available for 3 patients

Fig 2: Correlation between residual volume and number of courses of intravenous antibiotics in female CF patients

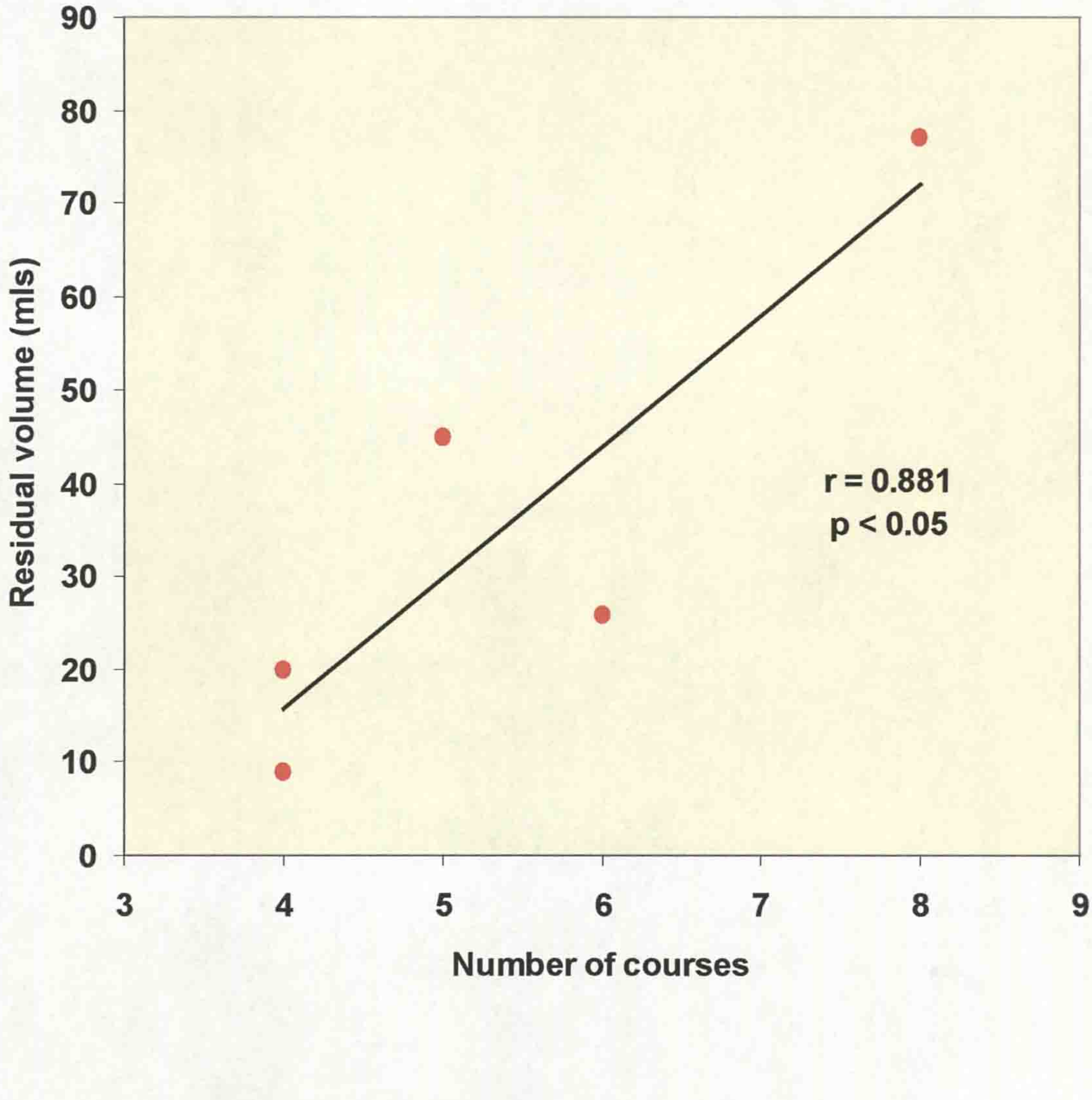


Fig 3: Correlation between residual volume and number of days on intravenous antibiotics in female CF patients

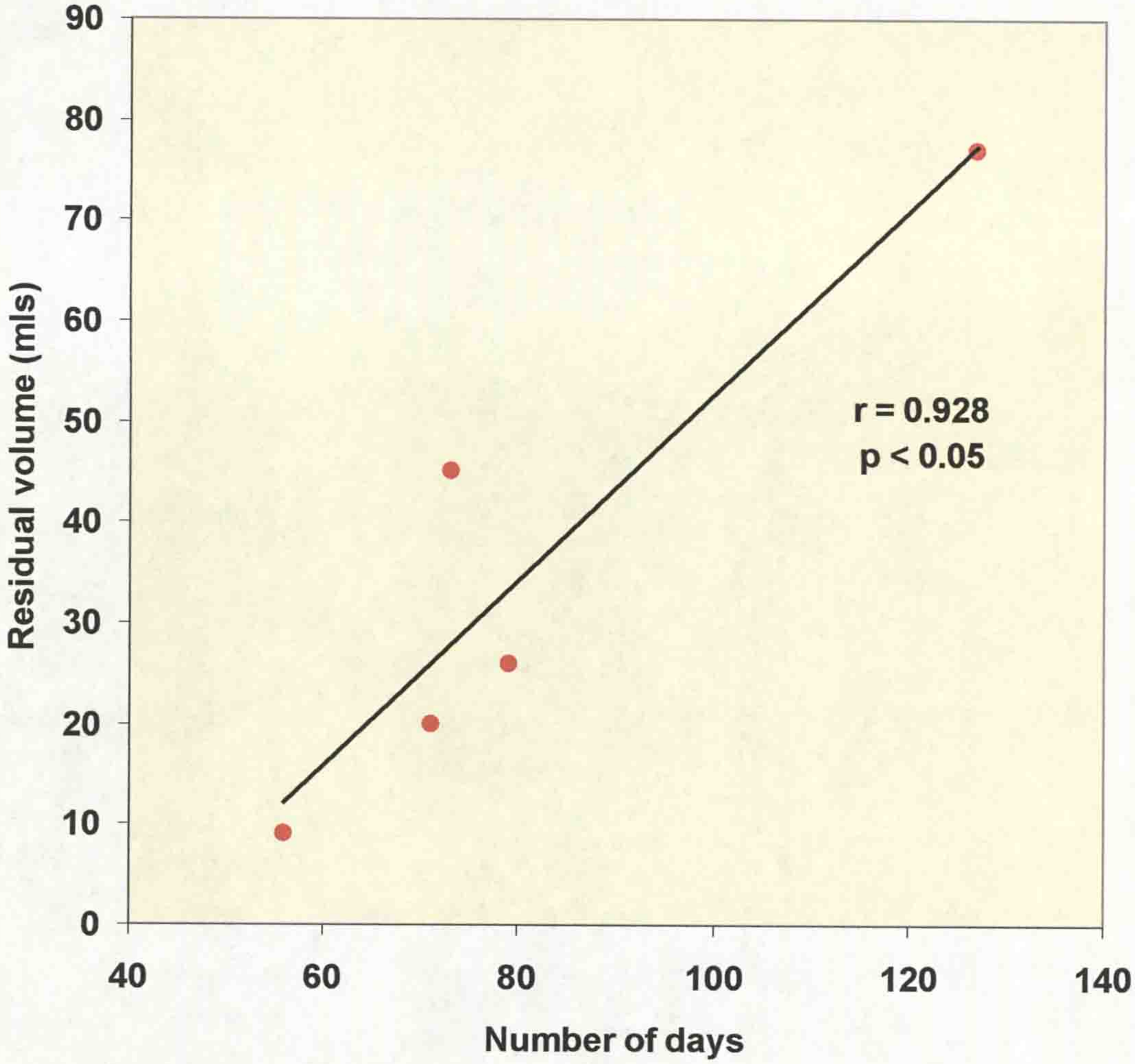
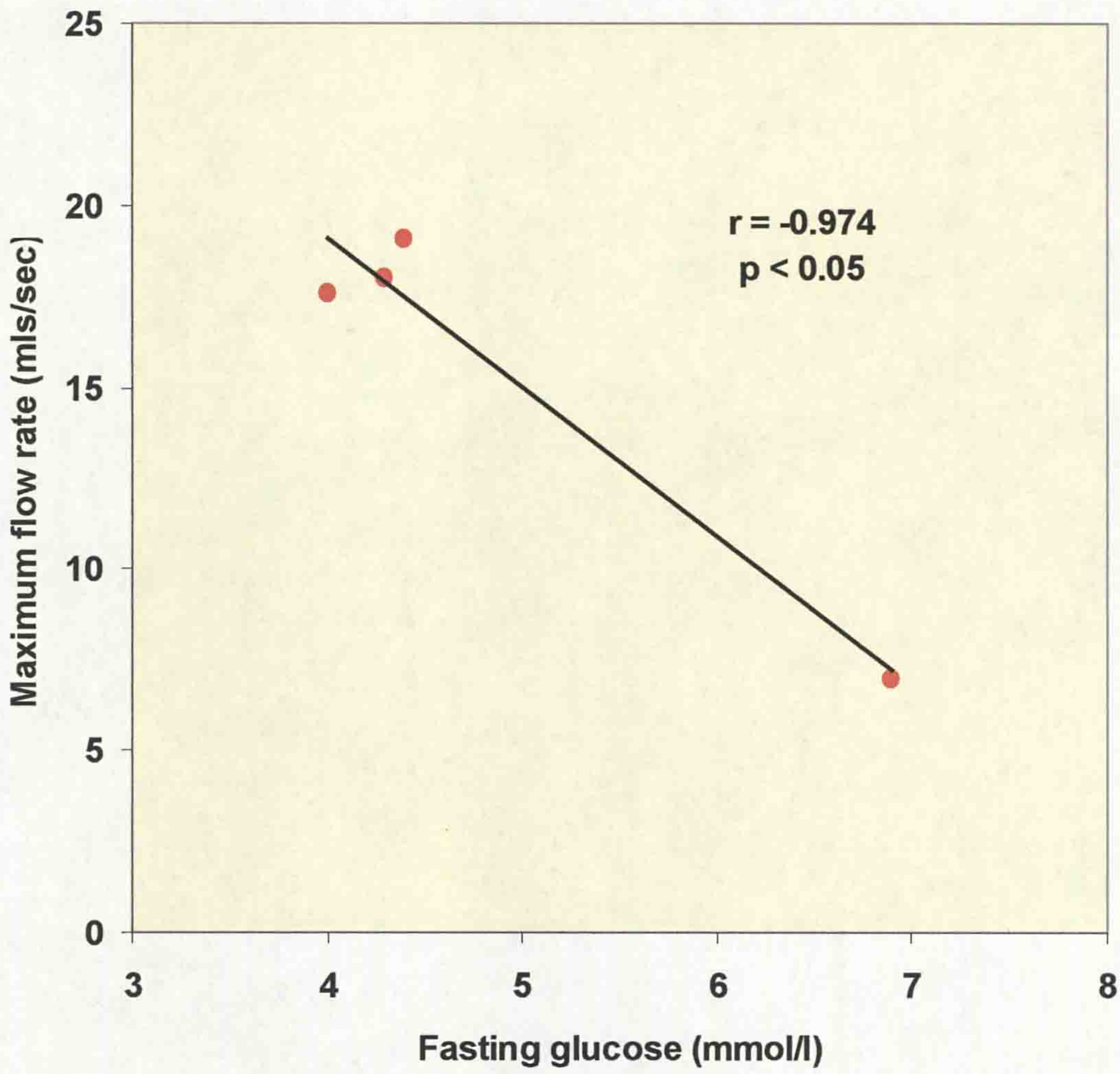


Fig 4: Correlation between maximum flow rate and fasting glucose in female CF patients



Summary of results

- The Liverpool nomogram may be used to define which centile a patient lies on when looking at the flow rates at a particular voided volume.

- My data shows that of those patients for whom full uroflowmetry data was available, 2 had average urine flow rates below the 10^{th} centile (defined as the lower limit of normality when looking at a normal population).

- There were positive correlations between residual volume and vitamin E levels and the requirement for intravenous antibiotic therapy. Maximum flow rate correlated negatively with fasting glucose concentration.

8:2 URINARY SYSTEM: DISCUSSION

Autonomic dysfunction involving the bladder has previously been investigated in conditions such as diabetes, multiple system atrophy and pure autonomic failure (see Chapter 1 Section 5:3). However, it has not previously been investigated in cystic fibrosis, although certainly urinary incontinence has been reported in this patient group. Orr et al (2001) showed that, on the basis of a semi-structured questionnaire, 68% of female CF patients had leakage; 64% of women aged 16 to 20 years complained of leakage compared to 12.8% in the general population (Chiarelli et al, 1999). In over 70% leakage was not confined to periods of exacerbation but did affect the ability to perform airway clearance and spirometry.

The clinical usefulness of urinary flow rates has previously been limited by the lack of absolute values defining normal limits. As flow rates are dependent on voided volume (Drach et al, 1979), these normal limits need to cover a wide range of voided volumes. Haylen et al (1989) compared maximum and average urine flow rates in normal male (age 16-64 years) and female subjects (age 16-63 years) with their respective voided volumes. Nomogram charts, in centile form, for both flow rates were constructed.

My study looked at female patients only. Haylen et al (1989) showed that in female subjects there was no relationship between urine flow rates and age or parity. A subsequent study by Haylen et al (1990) indicated that in women, the 10^{th} centile for maximum and average flow rates appeared to be a useful lower limit of normality to discriminate between those women unlikely to have voiding difficulties (centiles over 10) and those women at higher risk of voiding difficulties (centiles under 10).

My results showed that for those patients for whom maximum urine flow rate data and corresponding voided volume was available, 2 were on the 25th centile and 2 on the 10th centile. When looking at average flow rates and corresponding voided volumes, one patient was on the 50th centile and another on the 25th; the remaining 2 patients lay below the 10th centile indicating a significant abnormality. Therefore, despite the small numbers of patients included in my study, simple uroflowmetry has identified some abnormalities in urine flow, which in the absence of any identifiable mechanical obstruction may have a neurological basis.

Correlations

There was no relationship between any parameters of urine flow and spirometric or nutritional indices. Although very few patients took part in the uroflowmetry tests, there was a positive correlation between vitamin E levels and residual volume ($r=0.998$, $p<0.05$). This was an unexpected finding; vitamin E deficiency is associated with neurological dysfunction in CF (Sitrin et al, 1987), its replacement in diabetic patients results in an improvement in autonomic function (Manzella et al, 2001) and therefore if increasing residual volume implies parasympathetic damage, it would be logical to assume that as vitamin E levels increase, residual volume should decrease. However, vitamin E levels were all within the normal range in those patients for whom measurements were available.

Again, there was a positive correlation between residual volume and number of courses and number of days on intravenous antibiotics ($r=0.881$, $p<0.05$ and $r=0.928$, $p<0.05$ respectively) suggesting that the iller the patient, the greater the requirement for antibiotic therapy, resulting in a higher likelihood of autonomic neuropathy manifested as increased residual volume of urine. However, this should be interpreted with caution as 4 out of 5 patients had residual volumes less than 50 mls which is not thought to be abnormal (Betts and Fowler, 1992).

Maximum flow rate correlated negatively with random glucose concentrations ($r=-0.974$, $p<0.05$). Therefore, as glucose levels increased (in turn increasing the likelihood of autonomic nerve damage) so flow rates decreased possibly as a result

of reduced parasympathetic activity. None of these patients were known to be diabetic.

Clearly all these results need to be further substantiated using larger numbers of patients.

CHAPTER 9: CORRELATIONS ACROSS ALL SYSTEMS

Correlations existed only between spectral analysis and Ewing's tests parameters.

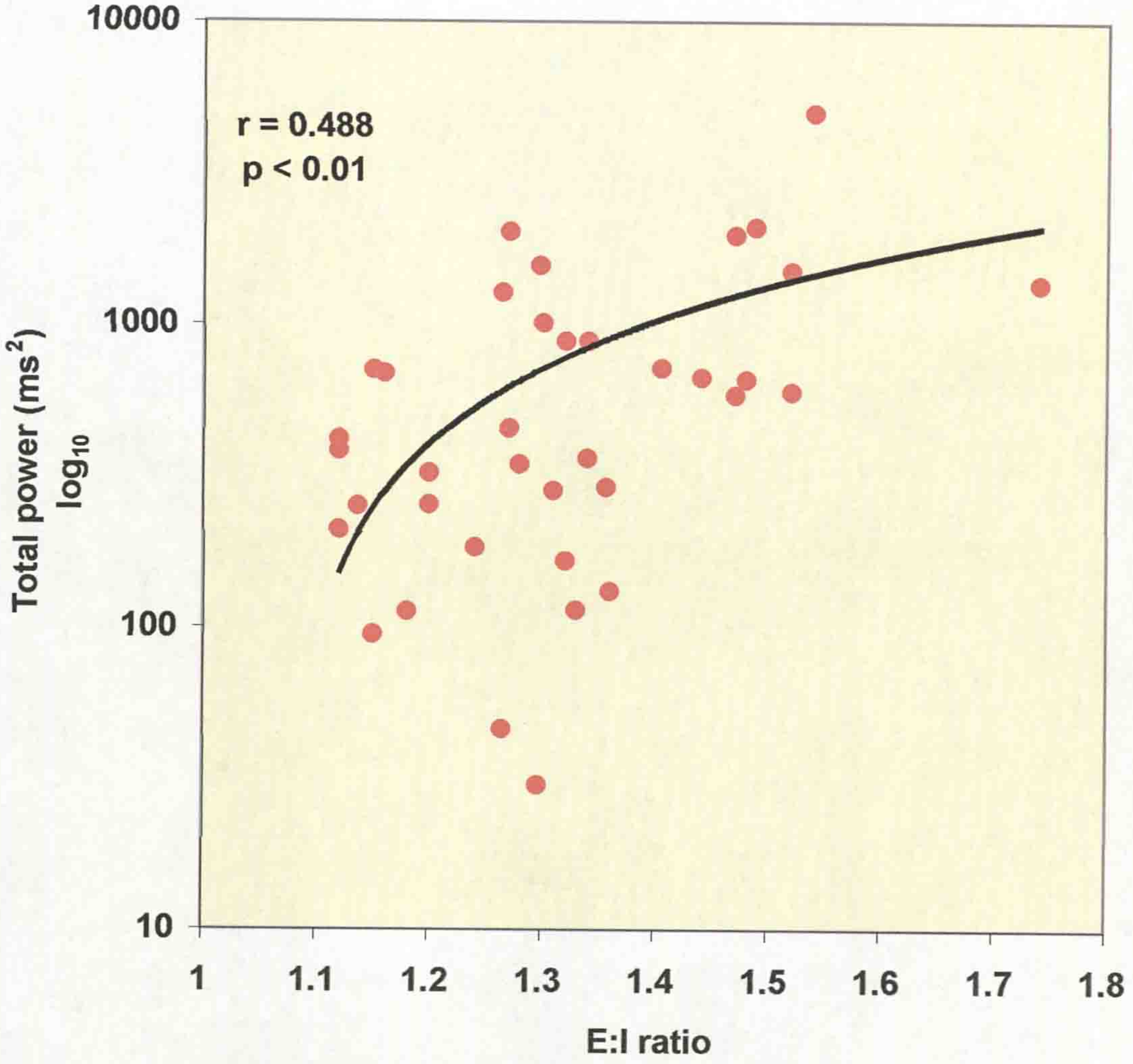
Total power correlated significantly with E:I and 30:15 ratios, both tests of parasympathetic function ($r=0.488$, $p<0.01$ and $r=0.649$, $p<0.001$ respectively) (Figs 1 and 2).

Once again, high frequency power (parasympathetic function) showed positive correlations with Ewing's tests of parasympathetic function (I-E, E:I and 30:15 ratios; $r=0.341$, $p<0.05$, $r=0.498$, $p<0.01$ and $r=0.582$, $p<0.001$) (Figs 3 to 5).

The low frequency (sympathetic) component of the power spectrum also correlated with parasympathetic tests (E:I and 30:15 ratios; $r=0.399$, $p<0.05$ and $r=0.625$, $p<0.001$) (Figs 6 and 7).

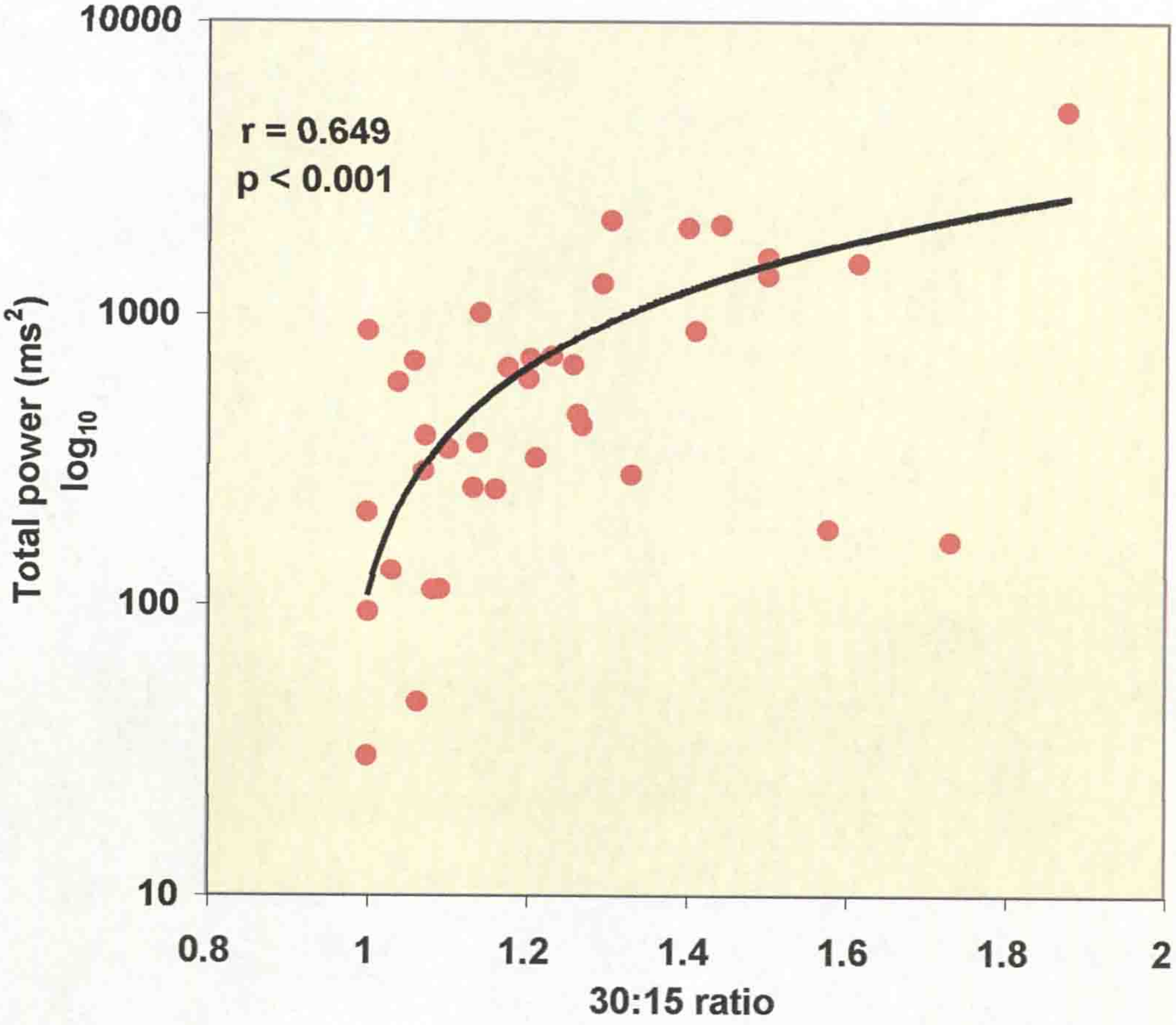
Global autonomic tone, or cumulative power, demonstrated significant correlations with I-E, E:I and 30:15 ratios ($r=0.362$, $p<0.05$, $r=0.547$, $p<0.001$, $r=0.641$, $p<0.001$) (Figs 8 to 10).

Fig 1: Correlation between total power (logarithmic scale) and E:I ratio in all CF patients



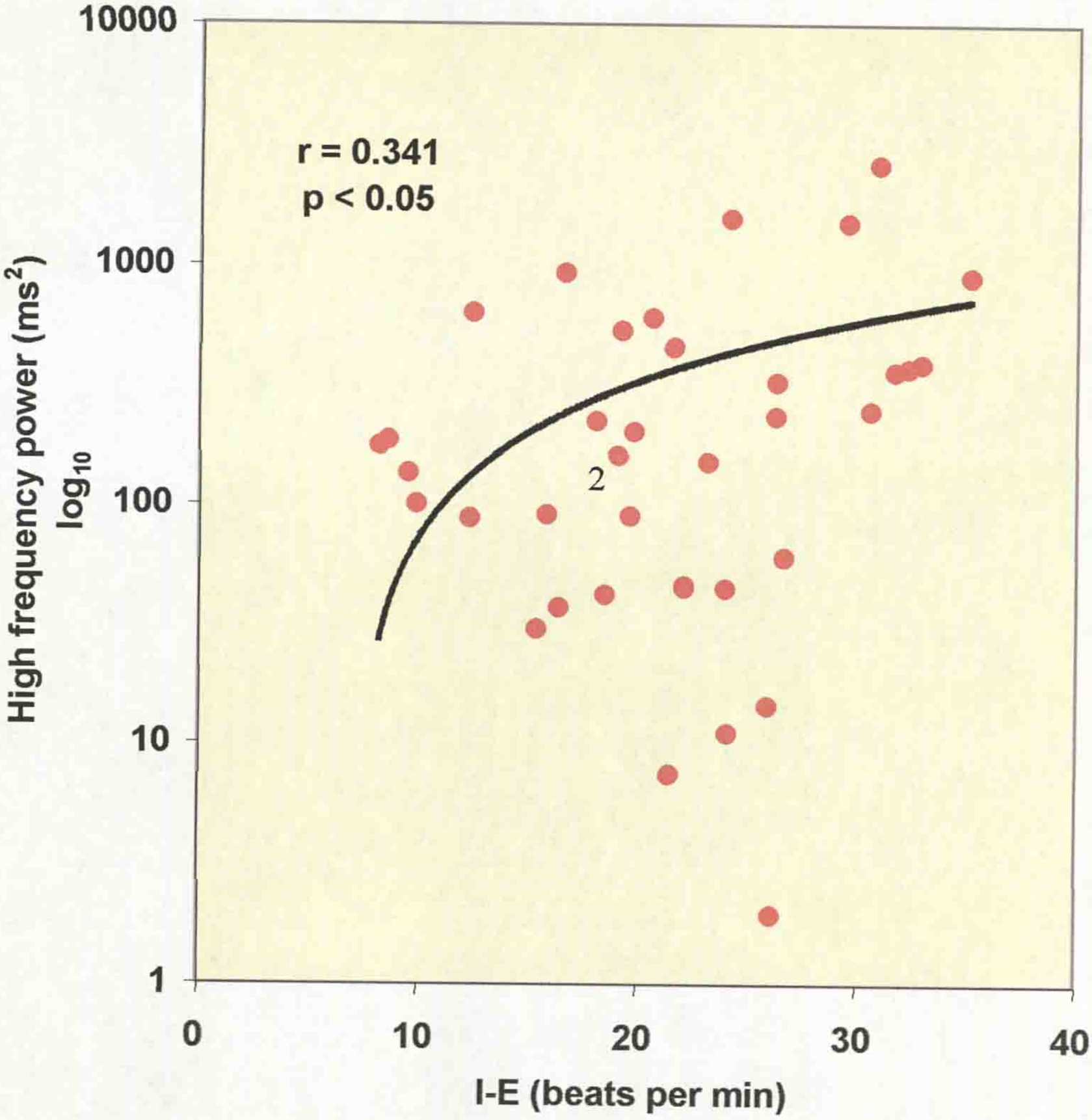
37 patients completed Ewing's tests and spectral analysis

Fig 2: Correlation between total power (logarithmic scale) and 30:15 ratio in all CF patients



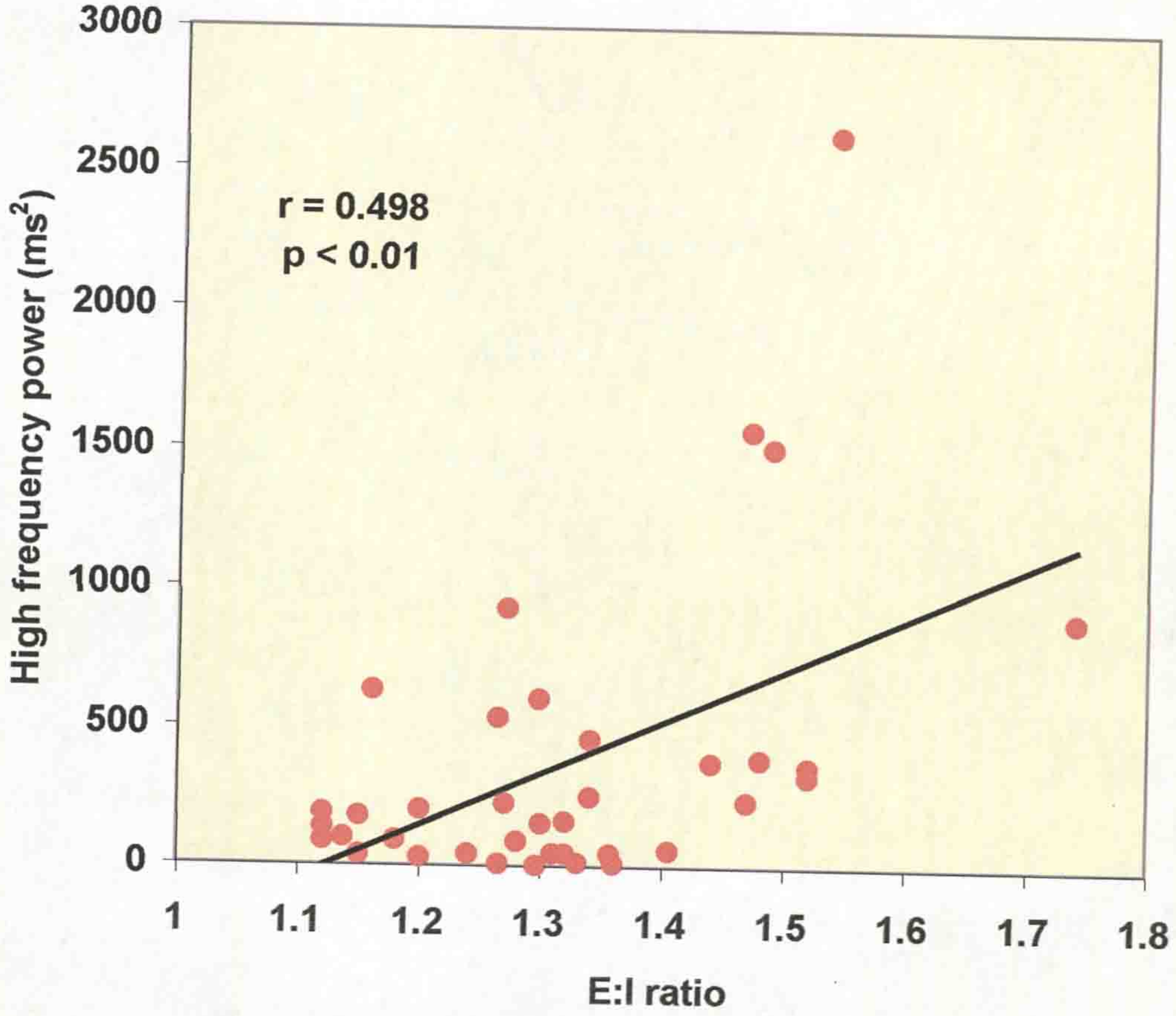
37 patients completed Ewing's tests and spectral analysis

Fig 3: Correlation between high frequency power (logarithmic scale) and I-E (both parasympathetic function) in all CF patients



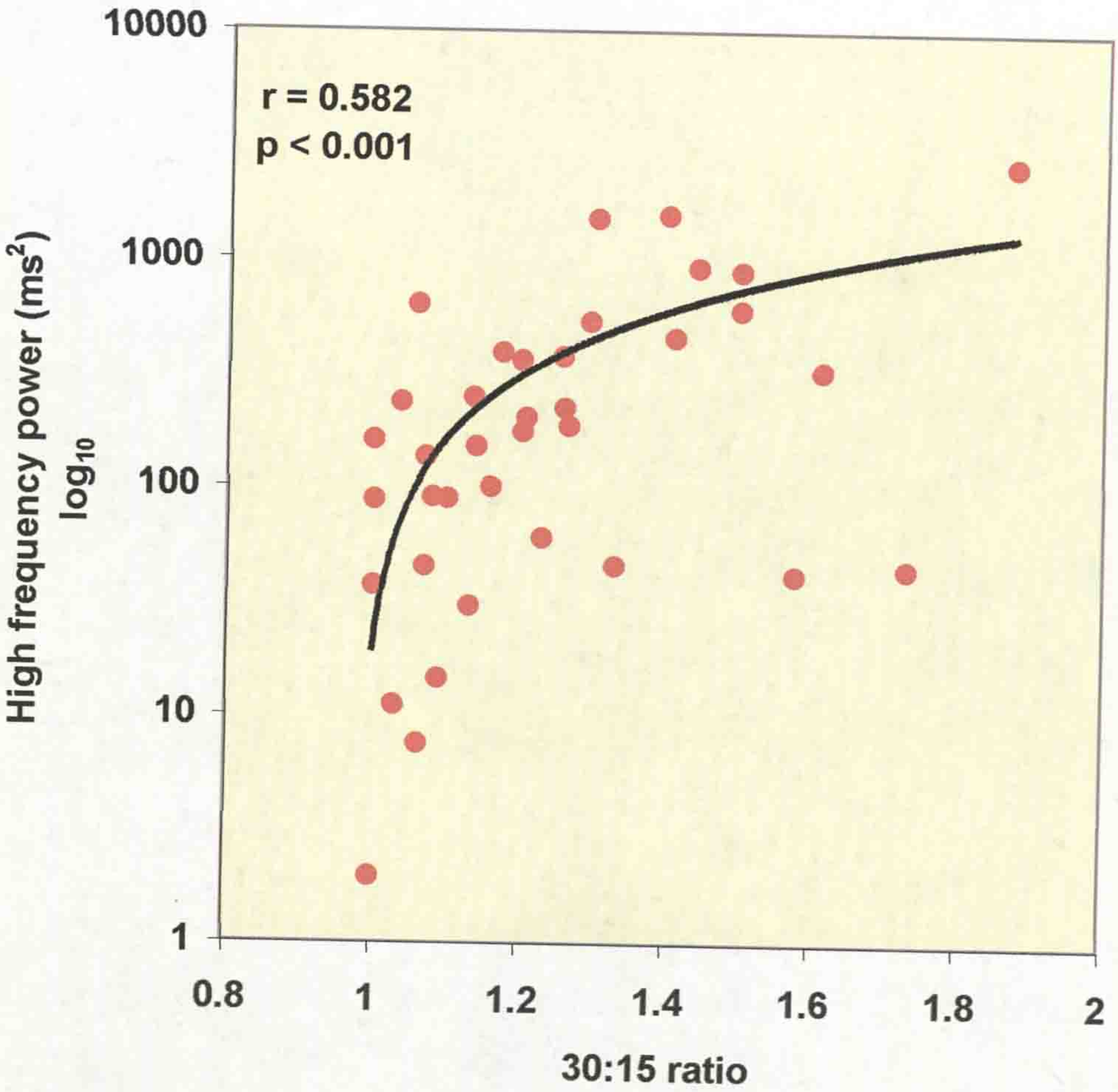
37 patients completed Ewing's tests and spectral analysis

Fig 4: Correlation between high frequency power and E:I ratio (parasympathetic function) in all CF patients



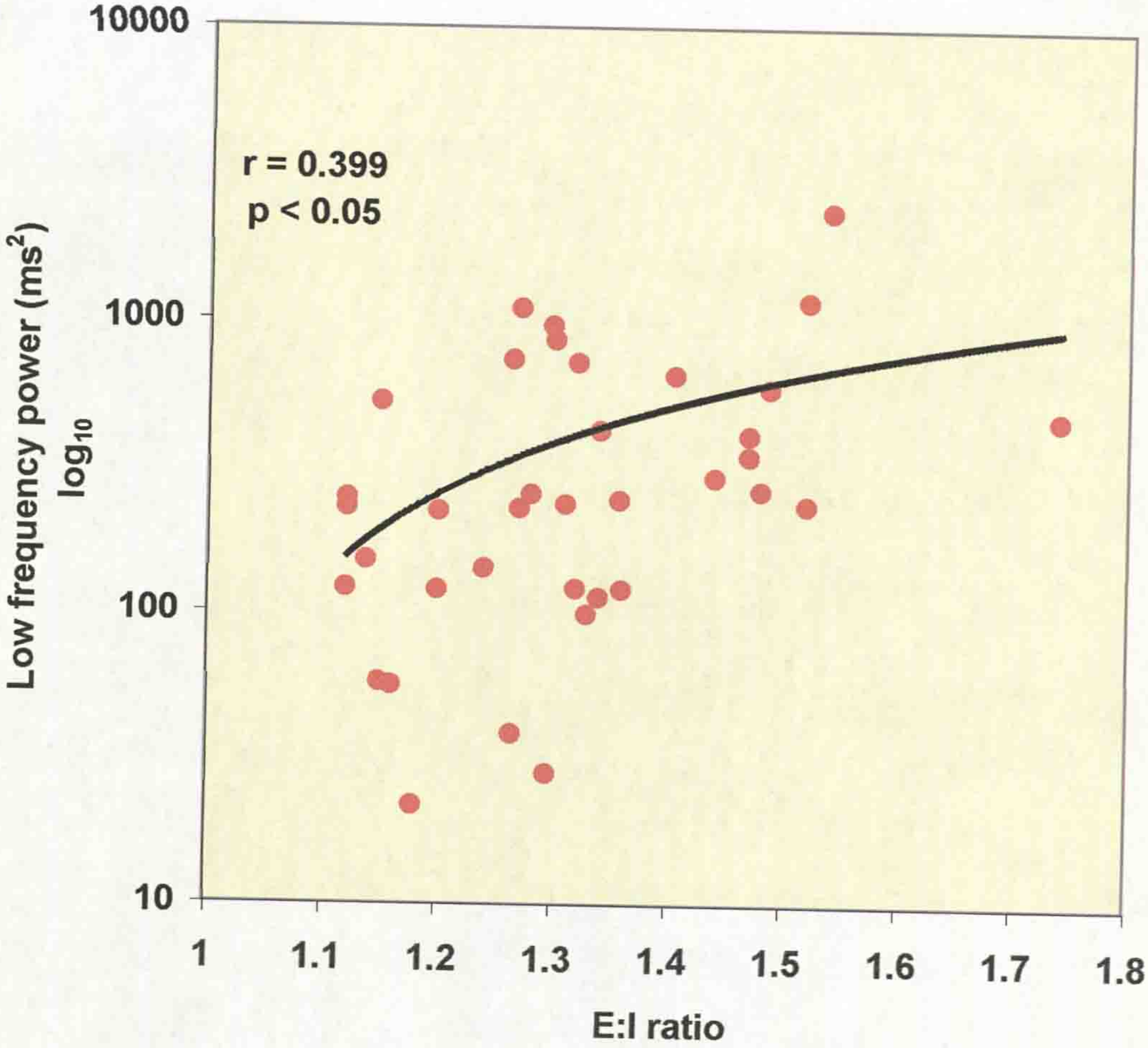
37 patients completed Ewing's tests and spectral analysis

Fig 5: Correlation between high frequency power (logarithmic scale) and 30:15 ratio (parasympathetic function) in all CF patients



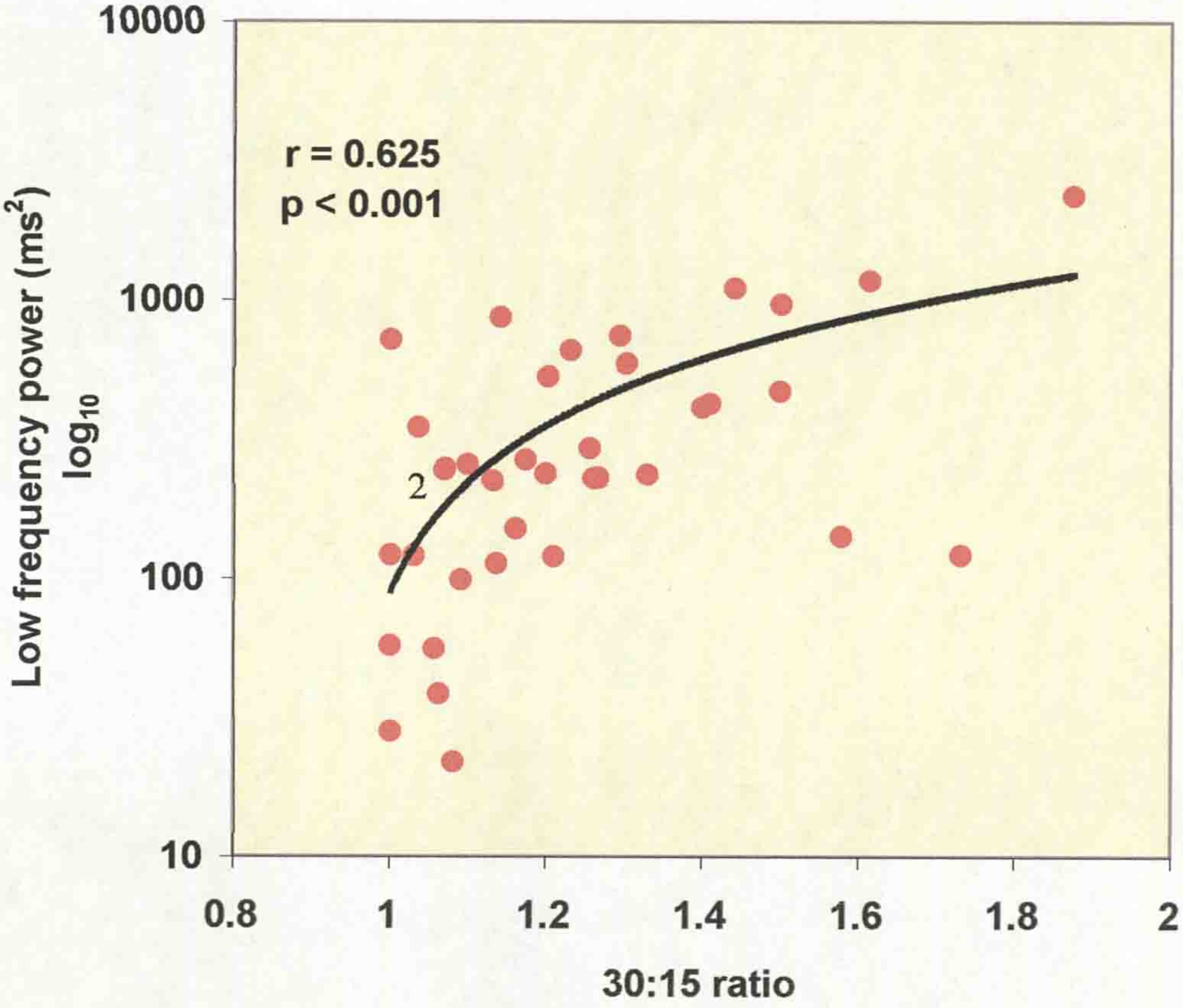
37 patients completed Ewing's tests and spectral analysis

Fig 6: Correlation between low frequency power (sympathetic function, logarithmic scale) and E:I ratio (parasympathetic function) in all CF patients



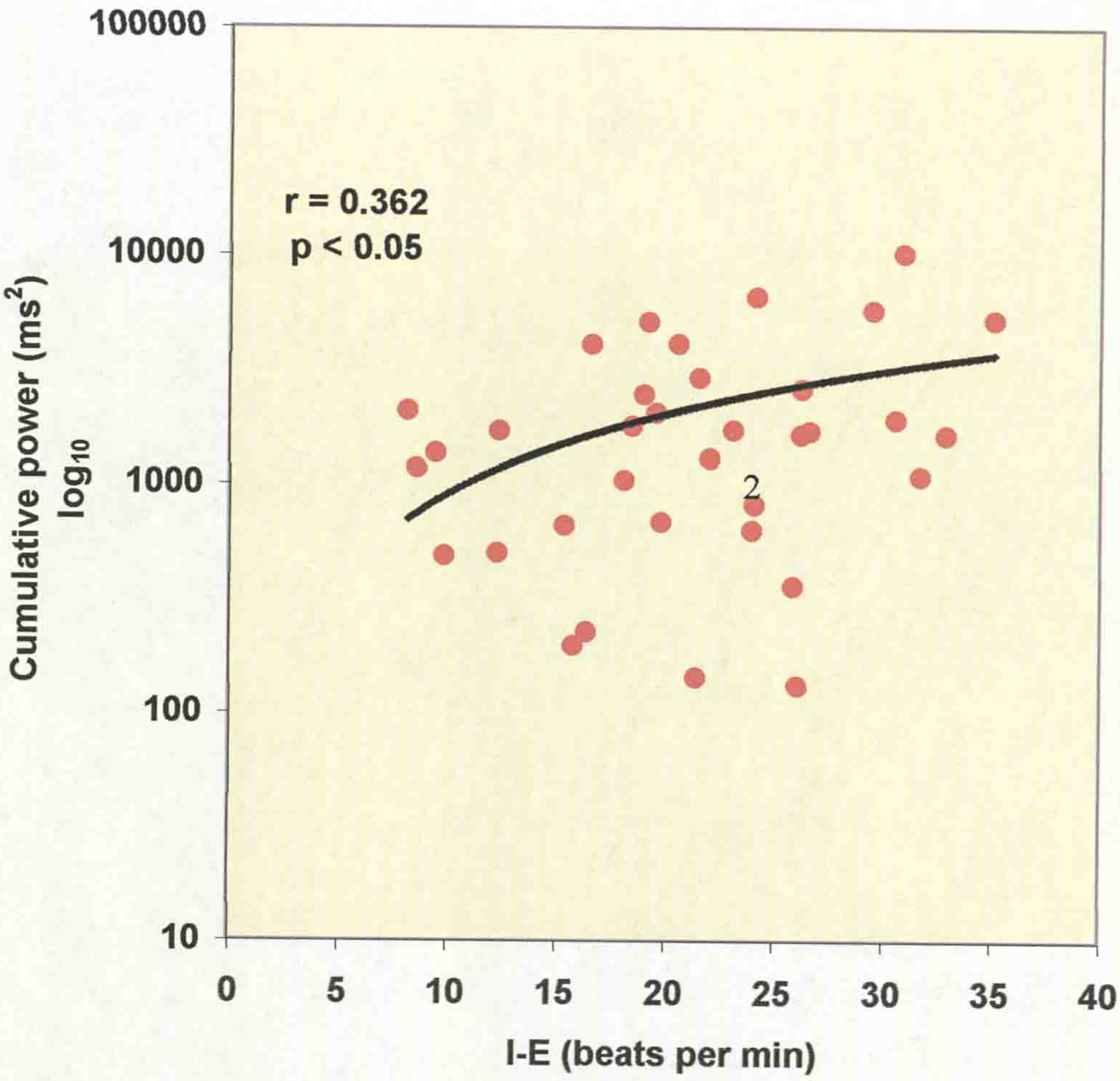
37 patients completed Ewing's tests and spectral analysis

Fig 7: Correlation between low frequency power (sympathetic function, logarithmic scale) and 30:15 ratio (parasympathetic function) in all CF patients



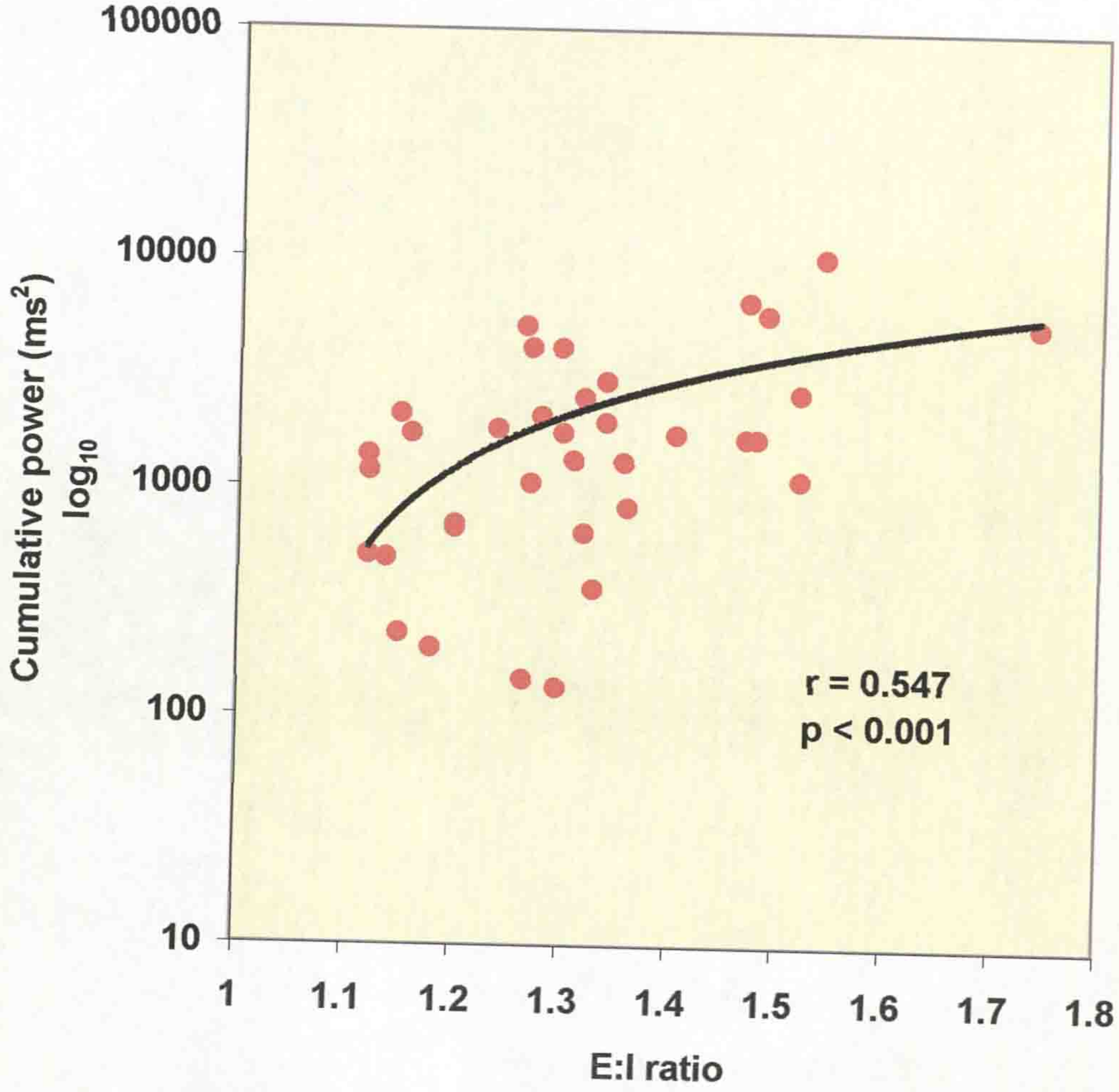
37 patients completed Ewing's tests and spectral analysis

Fig 8: Correlation between global autonomic tone (logarithmic scale) and I-E (parasympathetic function) in all CF patients



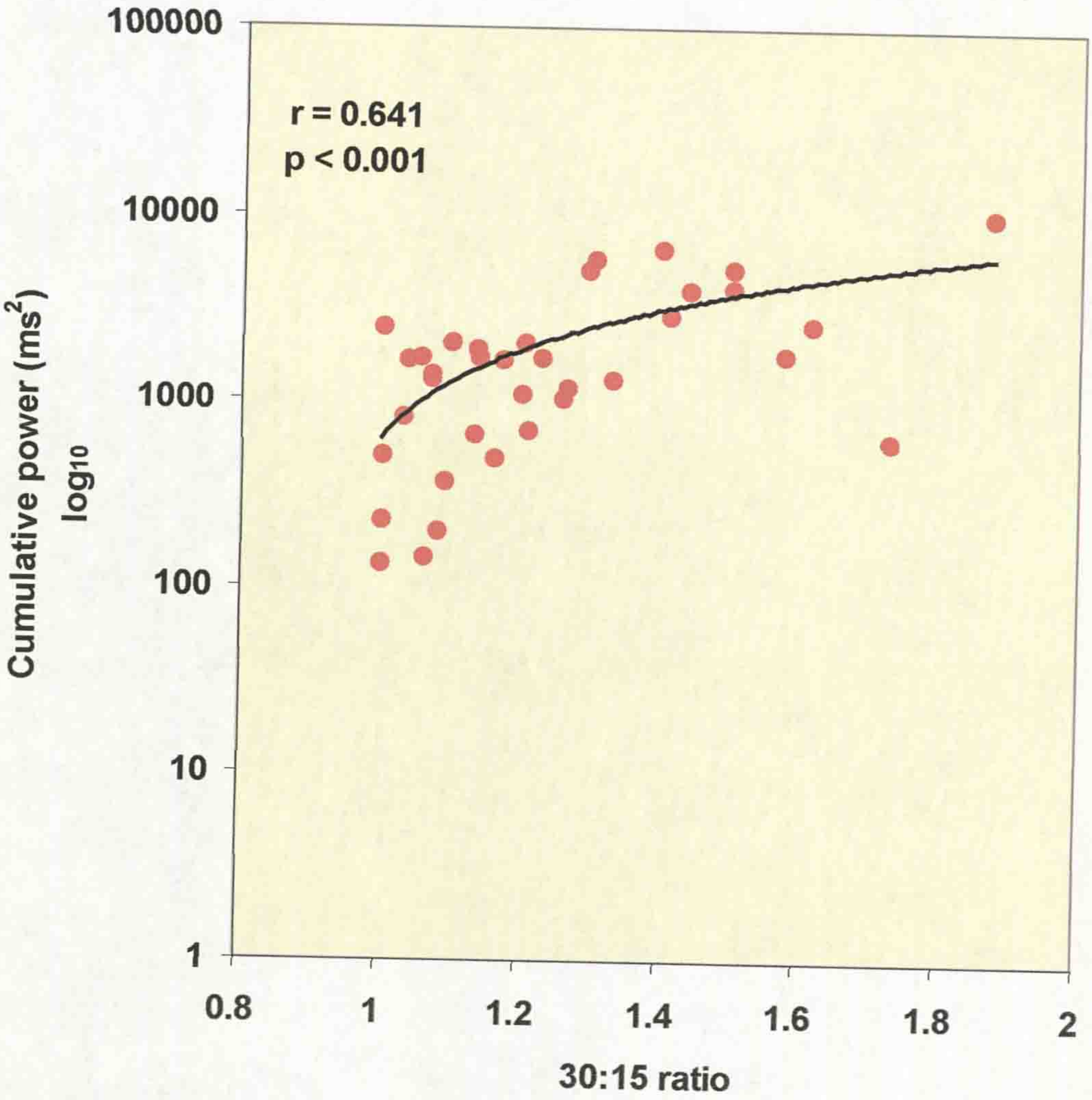
37 patients completed Ewing's tests and spectral analysis. However, due to computer failure, cumulative power was available for only 36

Fig 9: Correlation between global autonomic tone (logarithmic scale) and E:I ratio (parasympathetic function) in all CF patients



Although 37 patients completed Ewing's tests and spectral analysis, due to computer failure, cumulative power data was available for only 36 patients

Fig 10: **Correlation between global autonomic tone (logarithmic scale) and 30:15 ratio (parasympathetic function) in all CF patients**



Although 37 patients completed Ewing's tests and spectral analysis, due to computer failure, cumulative power data was available for only 36 patients

CHAPTER 10: SUMMARY/GENERAL DISCUSSION

Historically, the assessment of autonomic neuropathy has been based on a battery of 5 tests of cardiovascular autonomic function, first proposed by Ewing and Clarke in 1982. However, these are relatively insensitive to early changes in sympathetic function (Low et al, 1986). Furthermore, the sympathetic tests have poor reproducibility and are therefore much less reliable (Piha et al, 1991). The newer technique of power spectral analysis of heart rate variability has been used to assess autonomic function in several conditions such as diabetes (Lishner et al, 1987), chronic congestive cardiac failure (Saul et al, 1988), liver cirrhosis (Coelho et al, 2001) and uraemia (Vita et al, 1999). This technique has also for the first time demonstrated the presence of autonomic dysfunction in cystic fibrosis; Tattersall et al (2001) conducted a pilot study of autonomic function on 26 adult CF patients (mean age 25 years) and compared the results to the severity of their CF disease. There were strong correlations between total autonomic function and FEV1 ($r=0.62$, $p<0.001$) and FVC ($r=0.68$, $p<0.001$). These also correlated with sympathetic and parasympathetic activity, indicating that the autonomic nervous system becomes damaged with increasing disease severity.

Factors contributing towards autonomic dysfunction in CF have not previously been investigated, but of particular importance are metabolic mechanisms related to hyperglycaemia including activation of the polyol pathway (Greene et al, 1992), nerve ischaemia and hypoxia leading to lipid peroxidation of nerve membranes (Androne et al, 2000) and decreased nitric oxide synthesis (Stevens, 1995). There is also an association between Vitamin E deficiency and neurological dysfunction in CF, resulting in diminished reflexes, ataxia and decreased position and vibration sense (Sitrin et al, 1987). However, the effects on autonomic nervous systen function have not previously been reported. Fraser et al (1981) described autoantibodies against the β-receptor in 9 patients including one with CF. Compared to controls, these patients required higher concentrations of isoproterenol to increase pulse pressure by at least 22 mmHg (controls mean concentration 7.65 ng/kg/min versus patients 15.04 ng/kg/min, $p<0.001$) and to increase plasma cyclic AMP levels by at least 50% (controls mean 8.08 ng/kg/min versus patients 12.38 ng/kg/min, $p<0.02$).

The data in this study has proved that autonomic neuropathy exists in CF, certainly in the cardiovascular system. The results of Ewing's tests revealed that between 0 and 50% of CF patients had abnormal values (although the value of 50% was detected during the diastolic blood pressure change on isometric exercise, a test with a high coefficient of variation). Comparison with the control group showed that CF patients had significantly different mean values only for the 30:15 ratio (controls 1.48 versus patients 1.24, $p=0.004$) and systolic blood pressure change during orthostatic load (controls 14.5mmHg versus patients –0.2mmHg, $p<0.001$). In addition, microbiological status did not appear to influence the severity of autonomic dysfunction as identified using Ewing's tests.

My data shows that diabetic CF patients did not demonstrate any correlations between Ewing's tests and markers of disease severity. This may be explained by the fact that cystic fibrosis-related diabetes mellitus is distinct from conventional Type I and II diabetes (Hodson, 1992). However, there were significant positive correlations between the E:I and 30:15 ratios and pulmonary function in the patient group as a whole, as well as in non-diabetics, indicating that in CF diabetes appears not to contribute towards the development of autonomic neuropathy.

The technique of spectral analysis also indicated that a significant proportion of patients had abnormal values for each of total power (20.4%), high and low frequency power (30.6% and 12.2 % respectively), RR interval length (30%) and cumulative power (25%). This is consistently higher than for Ewing's tests. Furthermore, on comparison with control subjects, there were significant differences for all parameters (other than low frequency power). This suggests that again spectral analysis may be more sensitive than Ewing's tests. The finding that low frequency power did not differ between the groups suggests that sympathetic dysfunction occurs after parasympathetic dysfunction or that low frequency power does not discriminate effectively between the 2 groups. Similar findings have been reported by Lishner et al (1987) in their study of diabetics and by Saul et al (1988) in a group of patients with congestive cardiac failure.

The lack of difference in low frequency power between CF patients and controls was not due to nebuliser therapy in the former as a comparison between those patients who were taking regular nebulisers and those who were not failed to show any statistically significant differences in any of the spectral parameters.

My findings correlate with other recent studies which have shown that spectral analysis appears to be more sensitive than Ewing's tests at diagnosing autonomic neuropathy. Vita et al (1999) demonstrated that in chronic uraemic patients on periodic bicarbonate haemodialysis, the power spectrum of heart rate variability was a good discriminator of low and high frequency bands amongst uraemic patients with and without Ewing's test proven autonomic neuropathy. Takase et al (2002) performed 24 hour power spectral analysis and standard Ewing's tests on 18 diabetic patients. High frequency spectra were used as a direct measure of vagal nerve integrity from each hourly spectral plot. These values markedly decreased even in patients classified as having only early vagal damage on the basis of Ewing's tests.

A further interesting finding in my study has been the significant difference in spectral parameters between *B cepacia* and *Pseudomonas* colonised patients which Ewing's tests did not detect, again reflecting the greater sensitivity of the former technique in demonstrating that microbiological status may directly impact on the severity of autonomic dysfunction in CF.

My data has also shown a significant correlation between spirometric indices and parameters of spectral analysis in the patient group as a whole, substantiating work from an earlier pilot study by Tattersall et al (2001) that the autonomic nervous system becomes damaged with increasing disease severity in CF.

As with Ewing's tests, there were fewer correlations in the diabetics. However, as has previously been discussed, CFRDM is distinct from conventional Type I and II diabetes mellitus.

Although the data from my control study showed that the coefficients of variation of PD% were 2.6% and 3.9% for reproducibility and repeatability respectively, the lack of any significant difference in the PD% between controls and CF patients, or any correlations with markers of disease severity in this study possibly reflects earlier parasympathetic damage, and so if tests of parasympathetic pupillary function had been used then differences may have been revealed. Alternatively, as discussed earlier, PD% may be an insensitive test.

Despite complete uroflowmetry data being available for only 4 female patients, the use of the Liverpool nomogram (Haylen et al, 1989) did reveal that 2 of these patients had average urine flow rates below the 10^{th} centile, and that one of these had a raised residual volume (77 mls), indicating that in the absence of any mechanical obstruction in the urinary tract, this may have a neurological basis. This in turn may contribute towards urinary incontinence which is increasingly being recognised as a significant problem in CF (Orr et al, 2001).

When analysing bowel sounds, there were some significant differences between subject groups. For example, using spectral analysis there was a difference in median frequency between diabetics and non-diabetics and also when using CoolEdit, mean $\log_{10}$BS/minute differed significantly between controls and constipated patients, indicating that bowel sounds were more frequent in the latter; the number of bowel sounds decreased when patients were treated for their constipation. Furthermore, in the treated state bowel sounds were not significantly different from controls (mean BS/min treated state 5.34 versus controls 4.54, p=NS; mean $\log_{10}$BS/min treated state 0.11 versus controls 0.48, p=NS). Results analogous to these were demonstrated by Arnbjornsson (1986) who showed that 4 to 6 days postoperatively, patients who were previously obstructed had a pattern of bowel sounds similar to controls.

Finally, a correlation across the different systems was carried out. The only significant correlations existed between spectral parameters and Ewing's tests results. As expected, high frequency power correlated with tests of parasympathetic function (I-E, E:I and 30:15 ratios) (no correlation was found with the Valsalva ratio possibly because of an insufficient number of patients able to perform this manoeuvre). Similar observations were made by Freeman et al (1991) in a study of 15 insulin dependent diabetics. This showed that supine high frequency power was a significant predictor of I-E, E:I and 30:15 ratios. May and Arildsen (2000) also reported that E:I ratio, Valsalva and 30:15 ratios all correlated highly significantly with the mean log power of the high frequency band in the night-time during 24 hour Holter recordings in 136 diabetics (parasympathetic function predominates in the night-time).

My data has revealed that low frequency power did not correlate with sympathetic tests, reflecting the insensitivity or inaccuracy of the traditional tests. This has previously been observed by Freeman et al (1991) who demonstrated that the change in low frequency power on moving from a supine to an upright position was at best only a modest predictor of the systolic blood pressure change in response to postural change and a poor predictor of the diastolic blood pressure response to isometric exercise. The study by May and Arildsen (2000) decribed previously also revealed a relatively weak association between sympathetic function tests, particularly isometric exercise, and the log power of the low frequency band during daytime hours of a 24 hour Holter recording (accepted as an expression of sympathetic function).

The finding that low frequency power correlated well with the E:I ratio ($r=0.399$, $p<0.05$) and 30:15 ratio ($r=0.625$, $p<0.001$) was unexpected. One reason for this could be that while the power of the high frequency band is known to almost exclusively quantify parasympathetic activity, some investigators believe that the low frequency band is a measure of both sympathetic and parasympathetic activity (Akselrod et al, 1985).

Once again, total power and cumulative power (global autonomic tone) correlated with parasympathetic function tests, reflecting their greater sensitivity in comparison to sympathetic tests.

The lack of correlation between any of the cardiovascular tests and pupil diameter percent is at odds with the work of some previous investigators. For example,

Smith et al (1978) reported that almost all of 36 insulin dependent diabetics with autonomic neuropathy of the cardiovascular system had pupillary signs (sympathetic and parasympathetic testing). Martyn and Ewing (1986) showed that in 135 diabetic patients pupil cycle times (a parasympathetic test) became progressivley longer as the number of cardiovascular abnormalities increased. However, Schwingshandl et al (1993) found no association between PD%, light reflex amplitude or maximum constriction velocity and cardiovascular test results in 142 adolescents with Type I diabetes. This may be due to the young age group studied (range 10.4 to 19.8 years) in comparison to the previous 2 studies. My data also relates to a much younger age group.

The lack of association between cardiovascular tests and bowel sound data may reflect the poor reproducibility of the latter, particularly with CoolEdit. Therefore, despite the differences shown earlier between diabetic and non-diabetic patients and between constipated patients and controls, it would appear that the more invasive technique of manometry remains at this stage the best method for evaluation of bowel motility.

There were also no correlations with uroflowmetry data, although clearly a greater number of patients would be required. Alternatively, if the more accurate technique of filling and voiding cystometry been utilised then significant correlations may have been found. Certainly Ueda et al (1997) demonstrated that in 12 diabetic patients without sympathetic skin responses, residual urine volumes were greater and detrusor contraction pressures decreased as determined by cystometry.

Finally, the lack of correlations between the different systems in these CF patients may be a reflection of the 'patchy' nature of autonomic neuropathy, giving rise to the variability of clinical features. This has previously been demonstrated in diabetes where, for example, patients whose sole symptom of autonomic neuropathy is impotence rarely have abnormal cardiovascular tests: Ewing et al (1980) studied 73 diabetics with symptoms of autonomic neuropathy. 30 patients had impotence alone. These patients had normal autonomic function tests (responses to the Valsalva manoeuvre and sustained handgrip) whereas the majority with other symptoms eg postural hypotension, hypoglycaemic unawareness, sweating abnormalities and gastric fullness had abnormal results. In

addition, Calverley et al (1982) and Soler and Eagleton (1982) failed to demonstrate any differences in the ventilatory response to transient hypoxia during exercise between diabetics with and without cardiovascular autonomic neuropathy.

This study has demonstrated cardiovascular autonomic neuropathy in CF. Data also suggests that it may exist in the gastrointestinal and urinary systems, although clearly further work needs to be done in these areas. Nevertheless, when considering CF as a multisystem disease, autonomic dysfunction should be included.

CHAPTER 11: INDICATIONS for FUTURE RESEARCH

This study has shown that autonomic neuropathy exists in CF. However, several questions remain unanswered.

1. There is a need to examine the clinical relevance of abnormal cardiovascular responses in CF patients. Neumann and Schmid (1995) have already identified a statistically significant association between the degree of autonomic involvement and the presence of dizziness on standing, dysphagia, vomiting, faecal incontinence, urinary retention, gustatory sweating and numbness and hyperaesthesia of the feet or legs in diabetic patients. These symptoms can be extremely disabling. Autonomic neuropathy may be associated with an increase in the risk of sudden death in diabetics. Ewing et al (1991) observed that of 71 male diabetics under 60 years of age followed for 3 years, 13 had died, 8 unexpectedly. Of those with autonomic neuropathy, QT intervals were significantly longer in those who subsequently died, despite similar ages and duration of diabetes. None of the patients in my study reported any symptoms of autonomic neuropathy. However, with the expected increased survival of CF patients, the symptoms described above may become more apparent. Therefore, it may be possible to perform longitudinal studies of CF patients to look at the prevalence of abnormal cardiovascular test results as well as the development of symptomatic autonomic neuropathy. This is clinically relevant because certainly in diabetics Ewing et al (1980) showed that patients with symptoms of autonomic neuropathy and abnormal autonomic function tests had a calculated mortality rate after 2.5 years of 44% and after 5 years 56%.

2. My examination of the ophthalmic system in CF failed to reveal any differences in PD% between controls and patients. However, had tests of parasympathetic function been used, differences may have been observed. Therefore, there is a need to look at pupil cycle time and light reflex latency in CF patients.

3. The use of bowel sound analysis did not demonstrate any significant differences between patients and controls. This may have been because of the number of factors which can influence bowel sound recordings. Therefore, it would appear that manometry may provide the most accurate means of investigating gastrointestinal motility in CF. Further studies looking at oesophageal motility in CF and its relation to autonomic dysfunction are warranted.

4. Clearly larger numbers of patients than those included in my study are required to investigate the possibility of autonomic dysfunction in the urinary system. In addition, filling and voiding cystometry would provide more accurate, detailed information about detrusor muscle function.

5. Finally, the mechanisms of autonomic neuropathy in CF remain to be elucidated. My work suggests that CFRDM does not contribute towards this. However, CFRDM is distinct from conventional Type I and II diabetes mellitus. Vitamin E appears not to play an important role either, although many patients in my study had normal vitamin E levels.

Microbiological status may be important as I have shown in the spectral analysis section that those patients colonised with *Burkholderia cepacia* had worse autonomic function than those colonised with *Pseudomonas aeruginosa*. This was not due to any differences in markers of disease severity.

There exists the possibility of an immunological association with autonomic dysfunction. In diabetics with autonomic neuropathy, Gilbey et al (1986) found higher levels of circulating immune complexes than in controls. More recently, Vernino et al (2000) demonstrated that high levels of autoantibodies against nicotinic receptors in autonomic ganglia correlated with the severity of autonomic dyfunction in patients with a variety of dysautonomias. Antibodies against the β receptor have already been observed in a patient with CF (Fraser et al, 1981). This merits further work.

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APPENDICES

Appendix A: Data for Ewing's tests for CF patients

Subject	Diabetic	P/c	Av I-E	Av E:I	Av VR	30/15	Hgripdiff	Orthodiff
1	N	p	20.57	1.2975		1.5	20	15
2	N	c	16.4	1.15		1	13.3	0
3	N	c	19.2	1.264		1.293	30	0
4	N	p	30.9	1.54		1.875	18.3	-10
5	N	p	18.51	1.24		1.577	13.3	5
6	N	c	24	1.32		1.73	18.3	5
7	N	p	9.475	1.12		1.0714	15	10
8	N	p	9.87	1.137		1.16	15	5
9	N	p	31.7	1.52		1.2	10	-10
10	N	p	35.1	1.74	1.95	1.5	10	-10
11	N	p	21.45	1.265	1.26	1.0625	15	1.7
12	N	p	26.3	1.52		1.613	1.7	5
13	N	p	24	1		1	5	5
14	N	c	15.8	1.18		1.08	10	-2.5
15	N	p	26.65	1.405		1.23	3.3	-10
16	N	p	20.7	1.33		1.18	6.7	0
17	N	p	12.3	1.12		1	15	-3.3
18	N	p	8.2	1.15	1.07	1.203	10	-1.7
19	N	p	19.05	1.32		1	6.7	-10
20	N	p	25.9	1.33		1.09	20	5
21	N	p	8.6	1.12		1.267	18.3	8.3
22	N	p	12.4	1.16		1.057	0	-13.3
23	N	c	24.1	1.36		1.03	-6.7	0
24	N	p	21.6	1.34		1.41	0	10
25	N	p	16.6	1.27	1.635	1.44	6.7	11.7
26	Y	p	23.15	1.3		1.14	30	-1.7
27	Y	c	30.6	1.34		1.136	15	-10
28	Y	p	19.8	1.2	1.68	1.209	10	18.3
29	Y	p	32.9	1.48		1.174	20	-17.5
30	Y	c	32.3	1.44		1.256	20	1.8
31	Y	c	15.38	1.2		1.132	3.3	0
32	Y	c	22.1	1.357		1.07	10	0
33	Y	p	19.6	1.28	1.94	1.1	21.7	-15
34	Y	p	26.25	1.47	2.39	1.037	6.7	0
35	Y	c	18.1	1.27		1.261	10	5
36	Y	p	29.5	1.4875		1.3026	18.3	1.7
37	Y	c	26.1	1.296		1	0	3.3
38	Y	p	22.1	1.31		1.33	30	-10

Abbreviations for Appendix A

N	No
Y	Yes
P	*Pseudomonas aeruginosa* colonised patient
C	*Burkholderia cepacia* colonised patient
Av I-E	Average inspiratory-expiratory difference (beats per minute)
Av E:I	Average expiratory:inspiratory ratio (based on RR intervals)
Av VR	Average Valsalva ratio
Hgripdiff	Handgrip difference ie difference in diastolic pressure after and before isometric exercise(mmHg)
Orthodiff	Orthostatic difference ie difference in systolic pressure after and before standing upright (mmHg)

Appendix B: Data for Ewing's tests for control subjects

Subject	Av I-E	Av E:I	Av VR	30/15	Hgripdiff	Orthodiff
1	20.55	1.35	1.82	1.593	13.3	10
2	20.7	1.38	1.51	1.318	23.3	8.3
3	12.87	1.18	1.56	1.61	35	6.7
4	30.9	1.45	1.44	1.47	40	15
5	21.72	1.314	1.015	1.48	10	15
6	27.8	1.56	1.66	1.896	30	15
7	15.17	1.245	1.27	1.26	18.3	8.3
8	22.3	1.335	1.19	1.61	18.3	26.7
9	24.5	1.41	1.97	1.379	6.7	25
10	16.9	1.281	1.25	1.22	40	15

Abbreviations for Appendix B

Av I-E	Average inspiratory-expiratory difference (beats per minute)
Av E:I	Average expiratory:inspiratory ratio (based on RR intervals)
Av VR	Average Valsalva ratio
Hgripdiff	Handgrip difference ie difference in diastolic pressure after and before isometric exercise(mmHg)
Orthodiff	Orthostatic difference ie difference in systolic pressure after and before standing upright (mmHg)

Appendix C: Data for spectral analysis for CF patients

Subject	Diabetic	P/c	TOTP(L)	HF(L)	LF(L)	LF:HF(L)	RR (L)	Cum P
1	N	p	1561.69	601.8	959.91	1.594	0.93731	4076.4
2	N	p	293.418	74.94	218.48	2.9137	0.5913	747.49
3	N	p	1064.84	760.2	304.66	0.4057	0.69672	3860.5
4	N	c	94.6154	37.21	57.41	1.5443	0.60881	228.32
5	N	n	333.441	177.3	156.1	0.8814	0.66067	1067.3
6	N	p	1497.04	327.2	1169.9	3.5701		2612.2
7	N	c	177.025	30.82	146.21	4.7447	0.63221	403.13
8	N	p	1974.67	1565	409.21	0.2603		6574.1
9	N	c	112.495	90.67	21.828	0.2424	0.60078	198.59
10	N	p	1364.33	944.6	419.69	0.4483	0.7934	4479.8
11	N	p	705.058	176.1	528.93	3.0051		2069.3
12	N	c	1270.25	532.3	737.98	1.3937	0.85548	5064.5
13	N	p	4996.54	2623	2374	0.9054		10270
14	N	p	878.897	160.3	718.63	4.5182	0.76488	2461.4
15	N	p	183.631	41.86	141.77	3.3757	0.63258	1790
16	N	c	490.195	406	84.151	0.2078	0.7118	1780.6
17	N	p	416.432	186.9	229.56	1.23046	0.77146	1167.5
18	N	p	383.372	135.8	247.58	1.8253	0.73999	1369.5
19	N	p	686.733	630.6	56.178	0.0892	0.68134	1697.6
20	N	p	597.458	360.8	236.66	0.6572	0.65694	1095.3
21	N	p	878.32	455.3	423.56	0.9335	0.77095	2917.6
22	N	p	46.0474	7.477	38.471	5.1603	0.53947	145.01
23	N	c	165.685	44.7	120.98	2.7041	0.69671	630.65
24	Y	p	51.279	28.9	22.367	0.7756	0.64163	309.07
25	Y	p	307.644	70.3	237.35	3.4264	0.50937	937.63
26	Y	p	1011.7	151.5	860.17	5.6796	0.60574	1732.6
27	Y	c	362.253	248.9	113.31	0.456	0.63138	1927.4
28	Y	p	345.227	89.54	256	2.8822	0.72014	2042.8
29	Y	p	322.408	202.3	120.06	0.593	0.6726	685.08
30	Y	p	654.645	389.7	264.91	0.6884	0.74015	1653.8
31	Y	p	581.786	234.7	347.05	1.4803	0.72158	1654.3
32	N	p	251.205	100.4	150.8	1.505	0.78859	486.84
33	Y	c	452.675	224.2	228.45	1.02	0.59665	1039.9
34	Y	c	667.024	374.3	292.68	0.79094		
35	Y	p	2093.23	1503	589.97	0.3925		5772.1
36	Y	c	30.1074	1.946	28.161	14.331	0.5377	133.61
37	N	c	131.602	11.1	120.5	10.489	0.70181	819.55
38	Y	c	253.417	30.2	223.24	7.3975	0.62312	658.77
39	Y	p	281.733	45.92	235.81	5.1986		1308.6
40	Y	c	289.688	44.94	244.75	5.4475	0.67435	1297.8
41	Y	c	435.678	50.38	385.3	7.6481	0.67613	700.87
42	N	p	852.196	210.7	641.46	3.0403	0.70909	1656.8
43	N	p	113.487	14.46	99.031	6.8493	0.51833	362.34
44	N	p	1361	892.9	468.12	0.527	0.84748	5246.6
45	N	p	2025.68	924.6	1101.1	1.199	0.78212	4052.9
46	N	p	209.262	87.74	121.53	1.44154	0.67305	501.09
47	N	p	796.069	194.6	601.49	3.0905		1678.1
48	N	p	225.524	63.52	162.01	2.5537	0.62012	547.23
49	N	p	716.201	60.26	655.26	10.751		1720.6

Abbreviations for Appendix C

N	No
Y	Yes
P	*Pseudomonas aeruginosa* colonised patient
C	*Burkholderia cepacia* colonised patient
n	Patient colonised with neither
TOTP (L)	Total power in initial supine position (ms^2)
HF(L)	High frequency power in initial supine position (ms^2)
LF(L)	Low frequency power in initial supine position (ms^2)
LF:HF(L)	Low to high frequency ratio in initial supine position
RR (L)	Average RR interval in initial supine position (secs)
Cum P	Cumulative power (ms^2)

Appendix D: Repeatability data for spectral analysis parameters in 3 control subjects

(i) Total power (log_{10})

Subject	Day 1	Day 2	Day 3
1	2.711	2.911	2.843
2	2.959	3.078	3.095
3	3.051	3.356	3.459

(ii) High frequency power (log_{10})

Subject	Day 1	Day 2	Day 3
1	2.261	2.570	2.716
2	2.689	2..887	2.828
3	2.546	2.932	2.870

(iii) Low frequency power (log_{10})

Subject	Day 1	Day 2	Day 3
1	2.521	2.646	2.245
2	2.626	2.630	2.750
3	2.889	3.151	3.329

(iv) RR interval (secs)

Subject	Day 1	Day 2	Day 3
1	0.764	0.883	0.914
2	0.928	0.907	0.971
3	1.136	1.030	1.014

(v) Cumulative power (log_{10})

Subject	Day 1	Day 2	Day 3
1	3.375	3.330	3.468
2	3.418	3.658	3.505
3	3.583	3.721	3.805

Appendix E: Data for spectral analysis for control subjects

Subject	TOTP(L)	HF(L)	LF(L)	LF:HF(L)	RR(L)	Cum P
1	696.072	520.417	175.655	0.33965	0.9145	2938.988
2	1196.46	770.804	426.664	0.552351	0.907251	4551.28
3	508.69	374.343	134.347	0.355274	0.881582	2502.83
4	2271.36	855.288	1416.08	1.65803	1.03027	5258.388
5	2085.11	1699.45	385.652	0.226996	0.975358	5922.6
6	643.992	574.877	69.1156	0.122516	0.875496	2186.473
7	2307.81	2025.66	282.15	0.138175	0.919319	4615.092
8	203.116	29.556	173.56	5.79568	0.863025	735.648
9	196.931	105.213	91.718	0.872076	0.876761	624.137
10	6637.95	6495.44	142.51	0.021931	1.03856	13461.71
11	677.422	202.34	475.082	2.35041	0.746987	1908.228
12	908.265	836.602	71.6631	0.085671	0.760494	3371.775
13	316.88	204.831	112.049	0.548043	0.862781	932.591
14	5006.74	2330.62	2676.13	1.16093	0.966809	14603.73
15	987.996	770.964	217.033	0.281476	0.843705	2756.97
16	429.032	197.963	231.069	1.16584	0.641907	2158.03
17	2375.23	1587.75	787.482	0.495867	1.04304	5474.302
18	2465.55	1980.55	485.007	0.24483	0.993447	5406.414
19	342.679	217.438	125.242	0.576158	0.620372	1124.008
20	3515.3	1816.25	1699.05	0.938562	0.829669	7127.9
21	8168.33	7287.55	880.783	0.121383	0.976801	20831.95
22	4345.17	3789.73	555.443	0.146553	1.25375	15655.23
23	197.251	119.986	77.2645	0.646167	0.778346	781.447
24	473.661	303.84	169.821	0.561327	0.779032	1594.827
25	574.565	168.966	405.599	2.39847	0.917933	1398.593
26	971.4	819.966	151.434	0.184196	0.902402	3526.684
27	560.374	338.961	221.413	0.653206	0.850171	1602.233
28	1534.57	525.893	1008.67	1.9216	0.793727	3547.564
29	938.553	380.489	558.063	1.46996	0.867609	2920.18
30	570.067	308.251	261.816	0.849856	0.886432	1753.861
31	522.704	396.697	126.007	0.317962	0.727862	1339.002
32	373.431	121.906	251.525	2.06315	0.881617	1386.945
33	2051.37	724.233	1327.13	1.83143	0.946861	3867.717
34	2837.12	1127.73	1709.39	1.51594	1.02175	6087.53
35	3373.83	2380.32	993.501	0.417327	0.890488	9960.34
36	599.17	253.12	346.038	1.36753	0.827148	1761.948
37	601.476	300.254	301.222	0.994343	0.857144	1980.661
38	969.095	883.079	86.0158	0.097471	1.00524	2770.953

Abbreviations for Appendix E

TOTP (L)	Total power in initial supine position (ms^2)
HF(L)	High frequency power in initial supine position (ms^2)
LF(L)	Low frequency power in initial supine position (ms^2)
LF:HF(L)	Low to high frequency ratio in initial supine position
RR (L)	Average RR interval in initial supine position (secs)
Cum P	Cumulative power (ms^2)

Appendix F: Data for PD% for CF patients

Subject	Diabetic	P/c	PD%
1	N	P	69.56
2	N	C	64
3	Y	P	62.5
4	N	C	66.66
5	N	C	62.96
6	N	P	62.5
7	N	P	65
8	N	P	64.3
9	N	P	60.9
10	N	P	54.54
11	N	P	54.17
12	Y	P	50
13	Y	P	68.18
14	Y	P	66.7
15	Y	P	69.23
16	Y	C	70.8
17	N	P	58.33
18	N	P	53.8
19	Y	C	62.07
20	N	C	66.66
21	Y	P	68
22	Y	C	68
23	N	P	66.7
24	N	P	54.17
25	N	P	62.5
26	Y	P	66.66
27	N	P	69.56
28	Y	C	58.33
29	N	P	61
30	N	C	54.17
31	N	C	66.66
32	N	P	52
33	N	P	62.96
34	N	P	73.9
35	N	p	72.7

Abbreviations for Appendix F

N	No
Y	Yes
P	*Pseudomonas aeruginosa* colonised patient
C	*Burkholderia cepacia* colonised patient

Appendix G: Data for PD% for control subjects

Subject	PD%
1	50
2	66.67
3	63.25
4	67.39
5	58.33
6	62.5
7	66.62
8	54.55
9	63.77
10	58.33
11	54.35
12	53.85
13	59.17
14	56.83

Appendix H: Data for bowel sounds for CF patients

Subject	Diabetic	BS/min	Med Freq	Mean P
1	N	3.278	476.6	-21.8
2	Y	2.909	468.8	-26.5
3	Y	5.56	523.4	-23
4	N	9.126	351.6	-18.4
5	N	53.036	468.8	-24.3
6	N	0.691	414.1	-24.2
7	Y	4.368	445.3	-18.5
8	Y	2.425	492.2	-27.3
9	N	7.163	273.4	-23.5
10	Y	29.472	460.9	-18.5
11	Y	24.737	500	-24.5
12	N	8.088	476.6	-21.8
13	Y	8.471	476.6	-23.3
14	N	3.515	289.1	-21
15	N	6.263	476.6	-26.2
16	N	4.35	359.4	-21.3
17	N	2.553		

Abbreviations for Appendix H

N	No
Y	Yes
BS/min	Average number of bowel sounds per minute
Med Freq	Median frequency (Hz)
Mean P	Mean power (dB)

Appendix I: Data for bowel sounds for control subjects

Subject	BS/min	Med Freq	Mean P
1	0.921	445.3	-18.9
2	2.223	507.8	-26
3	14.113	500	-25.5
4	10.138	445.3	-19.2
5	1.65	500	-27
6		406.3	-20.8
7	6.52	515.6	-25.6
8	8.057	320.3	-22.2
9	0.828	492.2	-25.9
10	5.564	398.4	-17.2
11	1.04	484.4	-23.2
12	4.134	468.8	-22.6
13	3.081	492.2	-26.1
14	5.007	507.8	-27.8
15	0.591	226.6	-25.1
16	7.128	507.8	-26
17	1.596	515.6	-27.5
18		484.4	-22

Abbreviations for Appendix I

BS/min	Average number of bowel sounds per minute
Med Freq	Median frequency (Hz)
Mean P	Mean power (dB)

Appendix J: Data for uroflowmetry study on CF patients

Subject	RV	MFR	AVFR	VV
1	9			
2	20	18	9.1	282
3	26	7	2.5	31
4	45	19.1	13	174
5	77	17.6	8	262

Abbreviations for Appendix J

RV	Residual volume (mls)
MFR	Maximum flow rate (mls/min)
AVFR	Average flow rate (mls/min)
VV	Voided volume (mls)