AN INVESTIGATION OF THE ASSOCIATION BETWEEN TOXIN-PRODUCING STAPHYLOCOCCUS, BIOCHEMICAL CHANGES AND JAW MUSCLE PAIN.

A Thesis submitted to the Faculty of Dentistry, University of Sydney
for the degree of Doctor of Philosophy.

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December 1999

Declaration.

"I declare that this thesis does not contain any material which has been submitted by me previously for any degree or diploma to any University. To the best of my knowledge it does not contain any material previously published or written by another person, except where due reference is made in the text."

Neil McGregor: **Date:** 9-12-99

24 August 2001

ERRATUM

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As a maturity onset PHD student I have found this project to be one of the most fascinating and rewarding projects in which I have ever been involved. It was not a project I had to do, but a project I wanted to do. A search for the truth! The need to question the accepted paradigms, to look at what is really known, compared with what is assumed or interpreted. The need to question and evaluate my own thoughts and perceptions. A week did not go by without some exciting new insight, the need to once again review the literature and evolve my thoughts. Initially one attempts to predict the results, however the high frequency of the "*unexpected*" soon teaches one to assess the results for what they are and not what you think they are. This approach along with the high data collection per subject allowed the repetitive reassessment of the data leading to the evolution of a better understanding of the disease entity.

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CHAPTER 1.

1.1. Introduction.

Temporomandibular disorders (TMD) are chronic disturbances of the musculoskeletal system of the jaws involving muscle and joint disorders. Accepted TMD diagnostic criteria are termed the Research Diagnostic Criteria for Temporomandibular Disorders (RDC/TMD) as described by Le Resche et al (1992). These RDC/TMD criteria divide the investigation of patients into two axes. Axis I is a set of operational research diagnostic criteria for use in investigation of masticatory muscle pain, disc displacement, and degenerative changes of the temporomandibular joint (TMJ); Axis II is a set of operational research diagnostic criteria to assess chronic pain dysfunction, depression, non-specific physical symptoms, and orofacial disability. Table 1.1 summarises the RDC/TMD Axis I divisions for diagnosis of orofacial pain patients.

Table 1.1. TMD Research Diagnostic Criteria (Le Resche et al, 1992).

AXIS I	**Diagnostic Categories**
I	**Muscle Diagnoses**
	a. Myofascial pain.
	b. Myofascial pain with limited opening.
II.	**Disc Displacements**
	a. Disc displacement with reduction.
	b. Disc displacement without reduction, with limited opening.
	c. Disc displacement without reduction, without limited opening.
III.	**Arthralgia, Arthritis, Arthosis**
	a. Arthralgia.
	b. Osteoarthritis of the Temporomandibular joint (TMJ).
	c. Osteoarthrosis of the TMJ.
AXIS II	**Disability measures.**
	a. Pain intensity and disability.
	b. Depression (depression and vegetative symptom scales)
	c. Limitations related to mandibular functioning.

These diagnostic criteria provide a description of some of the physical and behavioural changes associated with TMD disorders but have limitations as they do not assess other body symptoms associated with TMD and the information does not address the aetiology.

1.2. Proposed Hypothetical Models of the Aetiology of TMD.

The first proposed aetiology of orofacial pain was that loss of posterior teeth resulted in collapse of the vertical dimensions of occlusion causing pressure on the eustachian tube, ear structures, the auriculotemporal nerve and the temporomandibular joint, with evocation of pain

(Prentiss, 1918). Costen (1934; 1936) popularised these concepts in his description of partially edentulous patients (posterior tooth loss), with marked mandibular overclosure, with associated impaired hearing, tinnitus, vertigo, pain in the area of the ear, trismus, occipital headache, burning sensations in the tongue, nose and sinuses. However, based on morbid anatomical assessment anatomists, Sicher (1948) and Zimmerman (1951) reported that the nerve impingement and eustachian tube blockage proposed by Prentiss (1918) and Costen (1934, 1936), was not anatomically possible. The nerve impingement hypothesis was developed by clinicians attempting to describe, explain and treat conditions where limited basic knowledge existed.

In his landmark paper, Schwartz (1955) introduced the term "temporomandibular joint-pain dysfunction syndrome". He described a syndrome with three phases: 1) an incoordination phase (minor muscle problems, TMJ clicks and/or recurrent TMJ subluxation); 2) a pain-limitation phase (painful spasm in any of the "antigravity type" muscles and limitations in mandibular movement); and 3) a limitation phase (muscle contractures which were not continually painful). He reported a female/male ratio of 4:1 and proposed that several factors influenced the outcome of the condition. These factors were termed: 1) predisposing factors (constitutional or physiological and temperamental or psychological); 2) contributing factors (occlusal anomalies and malocclusion); 3) precipitating factors (yawning, wide opening, long dental procedures and iatrogenic problems); and 4) aggravating factors (trauma, physiological and psychological influences). Possible aetiological factors were dental pathology, ear nose and throat diseases, adenopathy, neurological disease, rheumatoid arthritis, osteoarthritis, scleroderma, traumatic disorders and neoplasm. This paper was the first attempt to comprehensively describe and understand TMD.

Travell (1960) described in detail the pain referral patterns of the jaw and cervical muscles and the association between head and neck muscle pain. These associations have been summarised in "myofascial pain and dysfunction" (Travell & Simons, 1983) where chronic myalgic syndromes are described as the result of reflex feedback mechanisms between localised muscle "trigger points" and the central nervous system (CNS). The authors describe the many and various "trigger points" within individual muscles and their referred pain sites. They state "irritability of trigger points are influenced by the number and severity of perpetuating factors", such as mechanical stresses, nutritional inadequacies, metabolic and endocrine inadequacies, psychological factors, chronic infection, allergy, impaired sleep, radiculopathy and chronic visceral disease. Travell's studies have had a significant influence in understanding TMD and its management and introduced a medical assessment for the condition. Whilst Travell addresses medical conditions associated with TMD, the data presented is predominately from a clinical perspective and does not address associations between the medical problems and TMD symptom expression.

A significant change in diagnosis and management of TMD and orofacial pain followed the work of Ramjford (1961). In an uncontrolled study, Ramjford reported that occlusal adjustment in patients with "temporomandibular joint" symptoms resulted in reduced jaw muscle electromyographic activity and pain. He proposed that TMD arose as a functional disturbance, with differences between centric relation and centric occlusion and that mediotrusive contacts were the cause of increased muscle tone and parafunction. This had a profound effect upon clinicians and focused treatment methods based upon occlusal adjustment. Ramjford and Ash (1966) later qualified the conclusions, from the study, with a statement: "The role of occlusion and occlusal interferences in the cause of functional temporomandibular joint and muscle is controversial. According to recent investigations, patients (as a group) with functional disturbances of joints and muscles do not have any more occlusal interferences than individuals without the disturbances. On the other hand, such disturbances can unquestionably be eliminated in the overwhelming majority of cases by removal of occlusal interferences". Whilst Ramjford reported an association between the removal of occlusal problems and a reduction in symptoms, he recognised the resulting scientific dilemma where many clinicians interpreted the data as occlusal anomalies cause TMD (Geering, 1974; Vanderas, 1996). Subsequent studies by other authors (McCarroll et al, 1984; De Laat et al, 1985; Wanman & Agerberg, 1986; Egermark Eriksson et al, 1987; Meng et al, 1987; Dahl et al, 1988; Nielsen et al, 1989; Pullinger et al, 1992) have addressed this issue. These studies have reported that occlusal problems do not have a causal relationship with TMD when whole populations are studied. Other authors have associated occlusal problems with alterations in jaw muscle activity, which may influence pain (Sheikholeslam & Riise, 1983; Riise & Sheikholeslam, 1984). These data suggest that local functional changes associated with malocclusion are not aetiologically related to TMD, but may be associated with changes in symptom expression within patients with TMD. In support of the contention that local factors are not of aetiological significance, is that muscle pain is not restricted to the face but is associated with other symptoms including migraines and low back pain (Dixon, 1948; Berry, 1969; Carraro et al, 1969; Heloe, 1976; Weinberg and Lager, 1980; Eriksson et al, 1988; Turp et al, 1998).

Laskin (1969) introduced the term "myofascial pain-dysfunction syndrome" to describe a specific subgroup of TMD disorders and hypothesised that “masticatory muscle spasm” was the result of emotionally derived tension or stress. He suggested that "muscle spasm" was the primary cause of the pain rather than joint pathology and based this on the observation that radiographic evidence of joint pathology occurred in less then 5% of TMD patients. Laskin (1969) further suggested that emotionally derived tension or stress caused muscle fatigue as a result of prolonged "tension relieving" jaw activities. His hypothesis resulted in a shift toward the assessment of stress and TMD symptoms. Other authors suggested an association between stress and TMD (Schwartz, 1955; Yemm 1969a, 1969b; 1971a; 1971b; Fricton et al, 1985), however, recent studies (Schiffman et al, 1992; de Leeuw et al, 1994a; 1994b) have failed to identify a significant relationship between stressful events and TMD. In fact de Leeuw et al (1994b) found that stress was lower in patients with more severe symptoms and negative treatment outcomes. These data do not provide strong evidence linking stress with TMD.

Lund et al, (1991) proposed a model of pain adaptation, where patients have differences in normal masticatory activities which are attributed to the pain response. Molin et al (1972) showed that bite force was negatively correlated with levels of muscle pain and tenderness, whilst Bessette et al (1971) showed that alterations in chin tap electromyography (EMG) occurred in pain patients. Although these alterations in activity appear to be a response to pain, the results are heterogeneous and not found in all patients (Sharav et al, 1982; Zulqarnain et al, 1989). Increased spontaneous facial muscle EMG activity was first noted by Jarabak (1956) and again by Ramjford (1961). Munro (1975) found that unlike control subjects, 50% of TMD patients had spontaneous EMG activity whilst their mouth was open. This was the basis of the hyperactivity model for muscle pain (Travell & Simons, 1993). Sharav & Benoliel (1993) discussed the proposal that muscle hyperactivity may cause muscle pain especially the day following increased activity (Travell & Simons, 1993). However, Lund et al, (1991) concluded that muscle hyperactivity is not associated with chronic pain. Whilst muscle EMG hyperactivity does occur in muscle pain patients, it is likely that it results from the disease process and is not of aetiological significance.

Sessle (1992; 1995) described the central connections and neurobiology of pain responses in the trigeminal system. He proposed that alterations in the chemicals that modulate nociceptive transmission (substance P, enkephalin, serotonin and gamma-aminobutyrate) might play a role in excitation of pain responses. Neuroplasticity of the central and peripheral nervous system induced by inflammatory products or alterations in N-Methyl-D-Aspartate (NMDA) receptors in the brain has also been proposed as a mechanism for initiation of TMD pain (Dubner 1992; Mense 1993; Schaible & Grubb 1993). TMD dysfunction syndrome has been associated with phenothiazine medication (Evans, 1965; Hiatt

& Schwartz, 1966) and Pertoutka et al (1988) reported parafunction and/or trismus in the majority of users of the narcotic drug 3,4 methylenedioxymeth-amphetamine ("Ecstacy"). These data are highly suggestive of an association between a central nervous system dopaminergic anomaly and TMD. This is supported by the observation that tricyclic antidepressants, which increase noradrenaline activity, are associated with a reduction in TMD symptoms (Tura & Tura, 1990). Thus, the onset of TMD muscle pain may be associated with alterations in CNS dopamine associated changes. Examination of amino acid metabolism in TMD subjects is warranted as it may allow detection of alterations in the precursors of neurotransmitters and illuminate further avenues for research.

Diverse hypotheses have been proposed for the aetiology of TMD disorders. The dental literature is predominately of a mechanical-functional approach, which concentrates largely on the facial area, but has failed to identify the aetiology. The data reviewed in this chapter suggests that TMD is part of a systemic condition and may be associated with alterations in CNS nociceptive and neuronal plasticity changes that may be associated with dysregulated neurotransmission and inflammation. As a result a series of pilot studies were undertaken to investigate TMD from a systemic infection associated model.

1.3. Pilot study data.

Three pilot studies were conducted and reported as abstracts (McGregor et al, 1992a; 1992b; 1993a; 1993b; 1994a; 1994b; Zerbes 1993). These studies were firstly designed to assess pain distribution, factors related to onset or exacerbation, the prevalence of the associated signs and symptoms, the incidence of any muscle pain-associated condition and alterations in serum and urinary biochemistry compared with laboratory normal values. The second pilot study assessed the carriage of urinary tract staphylococcus whilst the third study assessed the toxicity of the staphylococcus obtained from the pain patients in a series of simple *in vitro* experiments.

1.3.1. First pilot study.

The first pilot study involved a questionnaire survey of 35 sequentially presenting RDC/TMD type 1a patients. Thirty-two females (age = 40.6±16 years; age range = 17-65 years; age of onset = 32.1±14; duration = 5-53 years) and 3 males (age = 49.7±13 years; age range = 38-73 years; age of onset = 45.3±15; duration = 32-67 years) completed the questionnaires and provided pathology tests from their medical practitioners. Twenty-three (66%) reported a gradual onset to their condition. Only 8% of subjects reported facial pain alone, whilst most patients had a pain pattern with a facio-scapulo-humeral distribution. Onset was reported to be associated with muscle fatigue (25 – 71%), stress (20 – 57%), trauma (8 – 23%), a genitourinary tract infection (6 – 17%), bells palsy (1 – 3%) and nasal surgery (1 – 3%). The predominant symptoms reported were parafunction (23 - 66%), sleep disturbances (22 - 63%), tinnitus (18 - 51%), gastroesophageal reflux (15 - 43%), chronic upper respiratory tract infections (13 - 37%), cardiac dysrhythmias (11 - 31%), skin dysesthesia (9 - 26%), aphthous ulceration (7 - 20%) and dermatitis or skin rashes (7 - 20%). Thus a strong suggestion of a wide-spread pain condition with multiple organ system involvement emerged, suggesting a systemic aetiology.

Known acquired or genetic diseases were found in 6 patients (17%). These diseases were diabetes mellitus (2 - 6%), sucrase-isomaltase deficiency (1 - 3%), myophosphorylase deficiency (1 - 3%), polycystic kidney (1 - 3%) and spina bifida (1 - 3%). These conditions were infrequently reported and their significance unknown but needed to be further investigated. Medical histories, obtained from medical records and patient histories, revealed a diagnosis and/or treatment for urinary tract infection (32 - 91%), measles (19 - 54%), mumps (15 - 43%), glandular fever (5 - 14%), giardiasis (2 - 6%) and dengue fever (1 - 3%). Eight of the 35 (23%) TMD patients reported investigation by urologists for repeated genitourinary tract symptoms - all with negative results under the accepted urology criteria (presence of haematuria, increased white cell count and bacterial counts $>10^6$ colony forming units per litre – Balows et al, 1991). Only urinary tract infections and glandular fever were reported to coincide with TMD onset.

A history of genitourinary infections was reported by 32 (91%) patients who had medical diagnosis of cystitis (19 - 54%) and/or renal infection (7 -20.0%); 4 patients who reported a history of genitourinary infections without medical diagnosis; low back pain was reported by 20 (60%) and irritable bowel by 9 (26%). Recurrent low-grade fever was reported by 10 (29%) TMD patients whilst 19 of the 32 females (59%) reported menstrual problems. These preliminary data suggested an association between infectious events, particularly genitourinary infectious events, and TMD symptoms.

If an infectious agent was involved, evidence of similar pain in the sexual partners of the TMD patients may give evidence of a potential transmissible agent. Seven of the 35 patients (20%) reported that onset of their condition occurred following establishment of a new sexual relationship, whilst 16 of the 19 (84%) TMD patients in long-term relationships, reported that their partners had chronic muscle pain. Thus, there was some evidence to suspect a transmissible pathogen may be involved in TMD conditions.

A biochemical assessment of 20 of the 32 patients was undertaken with each patient being assessed for alterations in standard serum chemistry (electrolytes, muscle enzymes) and 24-hour urinary electrolytes. All the serum measures were within the normal range however there was a reduction in 24-hour urinary sodium (79.9±19.9 – normal range 90-170 mmoles/L) and chloride (76.9±25.6 – normal range 170-250 mmoles/L) levels compared with the laboratory reference data. This suggests that an alteration in kidney function may be present.

1.3.2. Second pilot study.

As there was a history of urinary tract infection, with 23% of TMD subjects having multiple urological investigations, and evidence of a transmissible pathogen, a subgroup of 9 of the 35 TMD patients were assessed to determine if there was any alteration in their urinary tract microbial flora. These 9 TMD patients (age = 40.0±8 years; age of onset = 29.0±9 years; duration = 8.5±10 years; Female: Male ratio 8:1) were compared with 6 control subjects (age = 35.0±2 years; Female: Male ratio 4:2) who had no current muscle pain or fatigue. Three first of the morning urine samples were collected (urethral, midstream and last voided). Microscopic examinations for leukocytes and squamous cells were undertaken to assess pyuria and haematuria as evidence of infection. Microbial samples were cultured on blood layer and CLED agar plates in a humidified, CO_2 supplemented, incubator at 37°C. No subject had evidence of a current infectious event (haematuria or pyuria). Three of the 6 control subjects had no microbial growth whilst the remaining 3 had growth of diptheroids and/or *Lactobaccilus*. All TMD patients had heavy growths of *Staphylococcus spp.*. Bowel contaminant organisms *E.coli* and *Enterococcus spp.* were detected in 4 of the 9 TMD patients. *Staphylococcus epidermidis, Staphylococcus saprophyticus, Staphylococcus hominus* and *Staphylococcus haemolyticus* were isolated from the samples and are members of the Micrococcus subgroup 3, known entero-uropathogens. Thus the urethral microbial flora of the TMD patients was quite different from the pain-free control subjects. These data suggested that the increased staphylococcal carriage was linked to TMD.

1.3.3. Third pilot study.

Observation of the agar plates showed that many of the staphylococcal species isolated from the urinary tracts of the TMD patients were haemolytic, as they degraded the blood cells in the blood agar. Haemolysins are a group of membrane-damaging toxins produced by staphylococcal organisms. To assess the toxic characteristics of these organisms, supernatants from 12 different staphylococci obtained from the 9 TMD patients were acquired after overnight growth of the organisms in brain heart infusion broth (BHI). The supernatants were filtered to remove all bacteria and the filtrate was applied to chicken renal fibroblast cultures along with fresh BHI as a control. The cultures were observed microscopically for disruption of fibroblast morphology. Filtrate supernants from staphylococci that were haemolytic were observed to significantly disrupt the fibroblast morphology and in the cases of very haemolytic organisms, complete destruction of the fibroblasts was observed. The control BHI broth did not disrupt fibroblast morphology. These data suggested that the toxicity of the staphylococci may be associated with symptom expression in the TMD patients.

1.4. Pilot study conclusions.

RDC/TMD type 1a patients (Le Resche et al, 1992) appear to be:

1. A clinically discrete sub-component of more generalised muscle pain conditions such as fibromyalgia or myofascial pain syndrome.
2. Further, TMD symptoms occur in association with systemic multi-organ changes and do not appear to be a localised functional disorder.
3. There is a high-level reporting of a history, and signs and symptoms consistent with a gastro-genito-urinary condition, and evidence of a transmissible pathogen.
4. There was also evidence of a change in renal handling of electrolytes (sodium and chloride).
5. Heavy colonisation of urinary staphylococcal species was noted in the TMD patients.
6. There was no evidence of current urinary tract infections, but the staphylococcal species were observed to produce large quantities of membrane damaging toxins.

1.5. Hypothesis

The carriage of toxic organisms that produce lipid soluble toxins, such as staphylococci producing haemolysins, can induce muscle-associated TMD symptoms.

1.6. Aims.

The study aims to examine the hypothesis that toxic staphylococcus species can induce TMD associated muscle pain.

1. A group of TMD patients defined to have RDC/TMD type 1a facial muscle pain and an age- and sex-matched control group will be assessed. These study subjects will be acquired through clinical referral. This study will involve urinary amino and organic acid assessment as well as an assessment of staphylococcal toxicity.

2. A group of chronic fatigue syndrome patients will be studied collaboratively. Patients will be independently selected and assessed by medical specialists at Royal North Shore Hospital. The prevalence of TMD symptoms within this group will be assessed to see if the changes found in the first study are reproducible and unique to TMD. This study will include assessment of serum amino acids, antinuclear antibody (ANA) responses and standard blood chemistry, which will extend the assessments of the first study. This study will only assess the changes in CFS patients in association with TMD expression in an attempt to assess the homogeneity of TMD symptoms determined in the assessment of the defined RDC/TMD type 1a study (Study 1).
3. A third group of pain/fatigue subjects selected independently of the writer to assess the reproducibility of staphylococcal toxicity in relationship to body pain distribution and potential cytokine associations.

These data will allow the assessment of the association between symptoms, biochemical changes and bacterial toxicity. The independently selected study groups will provide objective assessment of the findings of the separate studies and will allow an assessment of the homogeneity of the changes associated with TMD symptoms in patients with other defined pain conditions.

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CHAPTER 2.

2. Onset, Pain Distribution, Symptom Prevalence and SCL-90-R Changes.

2.1. Introduction.

Chronic muscle pain conditions form a group of syndromes that have many similar characteristics. These heterogeneous yet overlapping groups of conditions may intermittently affect up to 70% of the population with severe forms, such as fibromyalgia, occurring between 5-10% (de Girolamo, 1991). These conditions represent a very common clinical and therapeutic problem (Magni et al, 1990; de Girolamo, 1991) with unknown aetiology. A number of hypotheses have been proposed to explain the complex aetiology, including psychological phenomena, neurotransmitter-related anomalies, reflex muscle hyperactivity, denervation and deafferentation-related occurrences, immunological- or cytokine-mediated events and viral infections (reviewed in Chapter 1; Vaeroy & Mersky, 1993).

Chapter 1 outlines the pilot study data of 35 chronic orofacial muscle pain patients, where an association was found with multi-organ symptoms and a history of urinary tract infection (McGregor et al, 1992). There was no increase in prevalence of reporting of viral infections such as measles, mumps or glandular fever. Initial assessment of the urinary tract microbiology revealed that although there was an increased prevalence of history of urinary tract infection the orofacial pain patients did not have pyuria or haematuria and therefore did not present with urinary tract infections. However, there was an increased prevalence of elevated midstream urinary staphylococcal counts ($P<0.02$) in the orofacial muscle pain patients (n=9) compared with age and sex matched controls (McGregor et al, 1993). As a result studies were commenced to assess the association between TMD symptoms and pain characteristics, Hopkins Symptom Checklist-90-Revised (SCL-90-R) responses, microbiology, whole body symptom prevalence and onset events.

This chapter describes pain distribution, symptom prevalence, psychological status and onset events in TMD patients. The study data was obtained from three distinct study populations. The first study group had defined RDC/TMD type 1(a) (Le Resche et al, 1992) who were examined by the author and the second study group had defined chronic fatigue syndrome (Sharpe et al, 1991) and were independently selected and examined by clinicians from the department of Endocrinology at the Royal North Shore Hospital, St. Leonards, Sydney. Both study populations were compared against their own age- and sex-matched control groups. Two study populations may allow identification of specific characteristics that are associated with chronic TMD symptom expression, as distinct from expression of other chronic pain conditions. If TMD symptoms present with a distinct aetiology or set of associations, these two study data sets should allow identification of common events. It is not the aim of this chapter to comment on CFS as a clinical entity but to assess TMD symptom expression and associations within a defined CFS population.

2.2. Literature Review.

2.2.1. Prevalence of TMD/Pain in the General Population.

Pullinger et al (1988) reported questionnaire responses from a non-patient population with jaw muscle pain, with pain reported by 14% with severe pain reported by 1%, with only 4% seeking treatment. On clinical examination, 48% reported at least one area of palpable muscle tenderness, including 13% with moderate and 19% with severe pain. The rank order of moderate to severe palpable muscle tenderness was lateral pterygoid (37%), superficial masseter (23%), temporalis muscle tendon (17%), deep masseter muscle (11%), anterior temporalis (10%), posterior temporalis (6%) and medial pterygoid (2%). Severe tenderness was most often found in the anterior and posterior temporalis. The frequency of symptoms (Pullinger et al, 1988) is similar to those reported in other studies (Heloe & Heloe, 1977; Nilner, 1981; de Laat & van Steenberghe, 1985; De Knater et al, 1993). Seligman et al (1988) noted that although clinically palpable muscle tenderness occurred in 48% of cases, moderate to severe tenderness occurred in only 6%. Eighty percent of those with palpable muscle tenderness were previously unaware of the tenderness and they concluded, "tenderness does not necessarily support the clinical presence of pain". Kleinknecht et al (1986) found that the difference between patient symptom reporting and clinical findings was related to the number and severity of the symptoms. Only 25% of patients with ≤ 3 symptoms reported muscle tenderness whilst 90% of patients with >3 symptoms reported muscle tenderness.

Thus, a number of studies indicate that between 44 and 48% of the population have clinically demonstrable TMD symptoms. Whilst between 14 and 21% of subjects are aware of TMD symptoms only 4 to 5% seek treatment. The degree of pain or discomfort appears to dictate patient reporting and treatment presentation. These data also suggest that the measurement of a single parameter such as palpable muscle tenderness, is not a reliable measure of other parameters such as pain intensity.

The three most commonly reported symptoms in TMD patients are muscle pain, muscle tenderness and muscle fatigue. Whilst there appears to be a poor association between muscle pain and muscle tenderness, a positive correlation has been described between muscle pain and patient-perceived muscle fatigue (Wanman & Agerberg, 1986a; 1986b; 1986c; 1986d; 1986e). In fibromyalgia patients, there was no difference in repetitive muscle activity, but there was a reduction in the ability to relax following muscle contraction (Elert et al, 1992). Similarly, paraspinal muscle did not fatigue differently in fibromyalgia patients compared with control subjects (Stokes et al, 1993) or indicate alteration in oxygen uptake with exercise (Sietsema et al, 1993). However there was an increase in reported pain (Sietsema et al, 1993) and a reduction in voluntary muscle endurance in fibromyalgia subjects compared with myofascial pain patients (Jacobsen et al, 1992). It can be seen from the above that the reporting of muscle fatigue by pain patients appears to be associated with increases in muscle pain levels and not a reduction in muscle contractile function. However a reduction in the ability of muscle to relax following repetitive contractions may be important in pain development.

2.2.2. Age and Sex Related Findings.

Szentpetery et al (1987) reported that as age increased there was an increase in TMD dysfunction signs (impaired mobility, TMJ-sounds), headache, parafunction, stress and prevalence of "mandibular dysfunction". The prevalence of headache was different in different age groups in males and females. Similar age related findings were noted in other studies (Fricton et al, 1985; Cooper et al, 1986; Grosfeld et al, 1986; Egermark Eriksson et al, 1987; Riolo et al, 1987). The most frequent age of reporting was 30 to 50 years with a decline following 50 years of age with a low incidence in early teens increasing in late teens and early 20's. Marciani et al (1985) assessed elderly patients in America and found that only 4% reported TMD pain. There is a general agreement that the degree, severity and frequency of TMD symptoms begin to increase slowly in the post-puberty period reach a peak in the 30-50 age group and subsequently decline after 50 years of age.

The female: male ratio between 3.5:1 and 4:1 is another consistent finding (Schwartz, 1955; Campbell, 1958; Pedersen & Hansen, 1987). Epidemiological data gives a clearer picture of the differences between males and females. Pullinger et al (1988) found that the more severe the symptoms the higher the female: male ratio. Seventy-eight percent of all subjects with palpable TMJ tenderness were female whilst 85% of those with moderate to severe tenderness were female. Of those subjects with palpable muscle tenderness, 68% were female and of those with multiple site tenderness, 87% were female (Pullinger et al, 1988). To further clarify the male to female incidence Wanman and Agerberg (1986a; 1986b; 1986c; 1986d; 1986e) noted that the ratio was 1:1 until approximately 18 years of age after which female incidence increased greater than males. Agerberg and Osterberg (1974) similarly reported a 1:1 ratio in individuals of 70 years of age. These studies show an increased female to male incidence from 18 to 70 years of age, typified by an increase in number of sites and severity of pain symptoms.

2.2.3. Other Symptoms.

Many studies have described pain in muscles other than the jaw in patients with orofacial pain and several have suggested that TMD is part of a generalised or systemic condition (Eggen, 1954; Forsberg, 1956; Turp et al, 1998). TMD patients have been reported to have muscular pain in the head (30%), neck (25-39%), shoulder (32%) and the back (14%) (Carraro et al, 1969; Weinberg and Lager, 1980). Turp et al, (1998) found that 19% of cases had pain limited to the distribution of the trigeminal nerve, 16% to the trigeminal nerve plus the spinal nerve dermatomes C2, C3 and C4, with the remaining 65% having pain outside these nerve dermatomes. Conversely, Heloe (1976) interviewed 27 individuals with lower back pain and noted that 37% also complained of TMD symptoms. Similarly a high proportion of patients with fibromyalgia have been found to report TMD pain (Dixon, 1948; Eriksson et al, 1988; Plesh et al, 1996). Plesh et al (1996) reported that 18% of patients selected to have TMD also could be diagnosed to have fibromyalgia whilst 75% of fibromyalgia patients had TMD symptoms.

Westling (1992) reported that TMD patients had an increased prevalence of joint hypermobility, which was associated with lower skin collagen levels in the TMD patients. Buckingham et al (1991) found that 54% of TMD patients had hypermobile joints of which 13% had Ehlers Danlos Syndrome (a genetic collagen cross-linking syndrome), and 5% were described as “Marfanoid” suggesting a possible Ehlers Danlos Syndrome-like condition. Thus 82% of those with joint hypermobility were unexplained suggesting accelerated cartilage destruction and a degenerative disease process.

In patients with fibromyalgia, a broad range of symptoms have been reported including: increases in sleep disturbance, fatigue, depression, spastic colon, mitral valve prolapse, bursitis, chondromalacia, constipation, diarrhoea, TMD, vertigo, sinus and thyroid problems, concentration problems, sensory symptoms, swollen glands, tinnitus, chronic cough, coccygeal and pelvic pain, tachycardia and weakness (Waylonis & Heck, 1992; Jennum et al, 1993), gastrointestinal symptoms (Mengshoel & Forre, 1993), muscle cramps and fasciculation (Jansen et al, 1992), cardiovascular symptoms (Visuri et al, 1992) as well as joint hypermobility (Gedalia et al, 1993). Stress was reported to be an aggravating factor by 21% of patients. Activities reported to aggravate the symptoms of fibromyalgia include computer work or typing (37%), prolonged sitting (37%), prolonged standing and walking (27%), heavy lifting and bending (19%) and repeated moving and lifting (18%). The occupations which correlated with a high percentage of fibromyalgia patients included general office workers, (20%), health care providers (14%) and teachers (11%) were not necessarily those individuals who performed these activities to the highest levels (Waylonis et al, 1994). Interestingly the jobs with increased prevalence of fibromyalgia appear to be high person to person contact jobs. This suggests that other factors such as transmissible pathogens may be involved.

2.2.4. Onset and Associated Disorders.

Factors associated with the onset of the TMD may also give significant information leading to discovery of the aetiological factors. Table 2.1. lists those factors reportedly associated with onset of painful TMD disorders.

Table 2.1. Diseases and Factors Associated with Onset of TMD Disorders.

Onset-associated Event	**Examples**
Trauma	Head and neck injuries in accidents Iatrogenic trauma - oral surgery, prolonged dental procedures
Orthodontic therapy	
Stress	
General anaesthesia	
Drugs	Phenothiazines, Neuroleptanalgesics, histamine, drugs of addiction
Overuse phenomena	Gum chewing, violin playing, scuba diving (mouth-piece clenching)
Associated diseases	
Neurological origin	Parkinson's disease, epilepsy
Endocrine origin	Acromegaly, diabetes, hypoglycaemia, hypo- hyper-thyroidism, hypo- hyper-parathyroidism
Immune functional alterations	Dermatomyositis, rheumatoid arthritis, Ankylosing Spondylitis, scleroderma, Psoriatic arthritis
Muscle origin	Myophosphorylase deficiency, myotonic dystrophy, Myotonia congentica, Myositis Ossificans
Infectious origin	Tetanus, Actinomycosis, Lyme disease
Other	Gout, chondrocalcinosis, tumours, chronic fatigue syndrome

No known cause or event was associated with onset of TMD by 36% of subjects (Weinberg & Lager, 1980). Fricton et al (1985) also noted that onset was not associated with any identifiable event by many patients and that the patients with no identifiable onset associated event were in the 66% of patients who reported a gradual onset to their TMD symptoms. These data suggest that a high proportion of TMD patients have a slow or gradual onset of symptoms without any identifiable onset associated event.

An association has been found between trauma (general anaesthesia, oral surgical procedures, motor vehicle accidents) and TMD (Schwartz, 1955; Taylor et al, 1968; Weinberg &

Lager, 1980; Fricton et al, 1985; Harkins & Marteney, 1985; Pullinger & Seligman, 1991). The prevalence of trauma varies considerably between the various studies; 4% (Schwartz, 1955), 9% (Taylor et al, 1968); 21% (Weinberg & Lager, 1980), 27% (Harkins & Marteney, 1985) and between 44% and 79% depending upon the type of TMD symptoms (Pullinger & Seligman, 1991). Harkins & Marteney (1985) reported that 26% of TMD patients reported a motor vehicle accident as the cause of their head or neck trauma of which 80% had cervical injury (whiplash). Of this group, 92% reported the trauma to be the precipitating factor of their "TMJ dysfunction". Pullinger & Seligman (1991) reported that a history of trauma occurred in between 44% - 79% of TMD patients, with the highest reporting in a subgroup with myalgia as their prominent symptom. However, studies examining the prevalence of TMD following car accidents and whiplash injury found very few cases of TMD associated with the accident (Heise et al, 1992; Propert et al, 1994). Interestingly, Magnusson (1995) found that most subjects who had post-accident whiplash pain could be diagnosed to have a chronic pain syndrome suggesting that the trauma may be an aggravating event for some underlying condition and not causative in nature.

Several studies have associated onset and exacerbation of TMD with stressful events (Schwartz, 1955; Laskin, 1969; Yemm 1969a; 1969b; 1969c; 1971a; 1971b; Banasik & Laskin, 1972; Evaskus & Laskin, 1972;). Stressful events at onset were reported by 25-26% (Weinberg & Lager, 1980; Fricton et al 1985). Using logistic regression analysis Schiffmann et al (1992) only found a weak relationship between stress and myalgic TMD patients. These data do associate stress with exacerbation and onset of TMD but the association is not strong.

Bell (1969) noted earlier reports that the onset of TMD dysfunction syndrome may be associated with phenothiazine medications (Lynch et al, 1961; Malkin, 1964; Evans, 1965; Hiatt & Schwartz, 1966; Kraak, 1967; Ryan & La Dow, 1968; Shapiro et al, 1965). These authors reported a range of effects including TMJ dislocation, hypotonia and dyskinetic movements of facial muscles. Turunen & Achte (1966) reported the onset of what he called the bucco-lingo-masticatory syndrome as a side effect of the treatment for neuroleptic syndrome and Sunden Kuronen et al (1983a) showed that neuroleptic drugs induce parafunction and dyskinesia in mice. Interestingly, Sunden Kuronen et al (1983b) showed that stimulation of dopaminergic neurones in the nigrostriatal subgroup of the basal ganglia resulted in increased masseter muscle tone. They suggested that this was a possible cause of increased jaw muscle tone and may be a significant factor in the initiation of TMD dysfunction. Dachi (1968) noted diabetes mellitus, epilepsy, narcotic addiction and chronic fatigue among his patient group. Interestingly, Pertoutka et al (1988) reported parafunction and/or trismus in the majority of users of the narcotic drug 3,4 methylenedioxymeth-amphetamine ("Ecstasy"). These data are highly suggestive of an association between a central nervous system dopaminergic anomaly and, TMD and jaw clenching. This is supported by the observation that tricyclic antidepressants, which increase noradrenaline activity, are associated with a reduction in TMD symptoms (Tura & Tura, 1990). Thus, a number of conditions may be associated with the onset of orofacial muscle pain that may be associated with alterations in CNS dopamine associated changes.

Oles (1978) also noted carbohydrate anomalies in five cases assessed by glucose tolerance tests. Associations have been made regarding the onset of TMJ symptoms in gum chewers, musicians (Fry, 1986; Hirsch et al, 1982; Kovero & Kononen, 1995) and scuba divers (Goldstein & Katz, 1982; Hobson, 1991). Hirsch et al (1982) showed that violin and viola players had oral symptoms similar to TMD pain patients, but found on electromyographic examination that there were no significant changes in latency or silent period to that normally seen in TMD disorders. They concluded that pain was related to mandibular position during playing, the pressure used when holding the instrument, the time played, and distinct from that of the generally accepted TMD. This possibly mimics the studies of Cox et al (1982), and Skiba and Laskin (1981) where experimentally induced fatiguing activity resulted in pain and EMG changes, which were not the same as seen in TMD. These findings are similar to those of Munro (1975) who noted that in patients with trauma-initiated "TMJ dysfunction" there was no silent period alteration. These data suggest that there may be a heterogeneous group of aetiological events and responses associated with TMD.

Assessment of onset of primary Fibromyalgia Syndrome (Bengtsson et al, 1986), revealed a similar incidence of onset factors as seen in TMD: 60% did not note any event associated with onset and 22% noted onset after trauma; and 18% developed symptoms after infections. Buchwald et al (1987) attributed the fibromyalgia syndrome to persistent Epstein-Barr virus (EBV) infection as 55% patients had an elevated antibody titre to EBV. Thus the events associated with onset of the systemic muscle pain conditions, such as fibromyalgia, do not appear to differ markedly from those assessed for TMD.

These studies indicate that whilst approximately 60-65% of pain patients report a gradual onset with no identifiable onset event, 35-40% report onset associated to infections, drug use, trauma and stressful situations.

2.2.5. SCL-90-R Scores and TMD.

The Research Diagnostic Criteria for Temporomandibular Disorders (RDC/TMD) requires the assessment of both physical (Axis I) and chronic pain dysfunction, depression, non-specific physical symptoms, and orofacial disability (Axis II). Table 1.1 (Chapter 1; page 1) shows the RDC/TMD Axis I divisions for diagnosis of orofacial pain patients. These features include pain intensity, SCL-90-R depression and somatization scores and the limitations related to mandibular functioning (Le Resche 1982). A psychophysiological model has been proposed for TMD and several studies have shown an association with increased psychological dimension changes, particularly depression scores, in TMD patients (Clark et al, 1985; Buckelew et al, 1986; Lee & Lee, 1989; Von Korff et al, 1988; Vimpari et al, 1995). The validity of the SCL-90-R data in pain patients, or the ability of the test to determine psychological events as distinct from physical symptoms has been previously questioned (Buckelew et al, 1986; Ray et al, 1992). Interestingly, other studies found increases in depression scores that were not in the psycho-pathological range (Wright et al, 1991). Therefore, these data suggest that the validity of the SCL-90-R depend on the clinical interpretation of reporting of both physical and psychological symptoms and the potential misinterpretation of a physical symptom to represent psychological dysfunction.

However, if drugs that alter dopamine, noradrenaline and adrenaline are associated with alterations in TMD expression (Lynch et al, 1961; Malkin, 1964; Shapiro et al, 1965; Turunen et al, 1966; Sunden Kuronen et al, 1983a; 1983b; Pertoutka et al, 1988) then a biochemical basis may exist for a combined physical/behavioural alteration. This data would support a psychophysiological model, but from a biochemical basis rather than an affective disorder.

2.2.6. Conclusions and Aims.

These data show that the investigations of pain patients have been reliant upon simple measures of muscle pain and clinically palpable tenderness associated with that pain. These studies have generally been restricted to the assessment of clinically defined groups of patients by specialised clinicians and few studies have appropriately examined the total symptom expression and multiple symptom reporting by chronic pain patients. Most studies have also concentrated upon a single or small group of symptoms that may occur as a result of a number of different disorders. This study will assess the relationship between body pain distribution, onset, systemic symptoms, SCL-90-R somatization and depression dimension scores and the occurrence of TMD symptoms in defined RDC/TMD patients and patients with diagnosed chronic fatigue syndrome.

2.3. METHODS.

2.3.1. RDC\TMD type 1a patients.

2.3.1.a. Patient and Control Selection.

Forty-six sequentially presenting patients, unrelated to the pilot study cohort, referred for assessment of chronic orofacial pain were interviewed and clinically examined over a seven month period. These patients were designated the chronic RDC/TMD type 1a patient group. Patients were selected as having muscle pain on the basis of a positive response on a visual analogue scale (VAS) of average pain intensity in the 2 weeks prior to consultation, the presence of palpable muscle pain in the reported pain areas and that pain was present on greater than 50% of days during the three months immediately preceding consultation. Pain was not associated with the teeth, TMJ clicking or arthritis and crepitation, sinusitis, salivary glands, nerve or vessel pathology. The data from this study population and the comparative control group has been reported in McGregor et al (1996d).

Forty-one age and sex matched control subjects were recruited. Nine were relatives of the pain patients and 32 were unrelated subjects to ensure similar socio-economic and ethnic backgrounds. Control subjects (C), were eligible for inclusion in the study if they had no response to the VAS of average pain intensity in the 2 weeks prior to consultation, did not give a history of chronic pain and had not sort professional advice or treatment for chronic muscle pain in the previous 12 months. Acute pain associated with trauma during the preceding 12 months was not an exclusionary criterion. Each patient and control subject provided informed consent and was assessed by the author.

2.3.1.b. CPRU Questionnaire.

To facilitate standardised data collection a comprehensive questionnaire (Collaborative Pain Research Unit Questionnaire ©1993-1995; CPRU) was completed by all study participants at initial presentation (Appendix 1). The questionnaire was compiled from an existing University of Sydney Orofacial Pain Questionnaire, together with features of other muscle pain conditions (generalised, regional and localised pain syndromes) reported in multiple articles from the literature (Medline, 1966-1991). The questionnaire contained: 1) four body diagrams (left and right head and neck, front and back of whole body for pain distribution); and 2) 154 questions on medical and family history, onset, exacerbation, signs and symptoms, duration, pain severity, previous treatment and potential aetiological conditions. On the body diagrams patients were asked to delineated the usual areas of pain, irrespective of their perception of its origin, but to qualify by notes what they perceived to be the origin if they though it was unrelated to their orofacial pain.

2.3.1.c. SCL-90-R Questionnaire.

Study participants completed an SCL-90-R and the prevalence and degree of responses to individual questions, the index and dimension scores were compared between the chronic RDC/TMD type 1a patients and control (C) groups. The SCL-90-R raw dimension scores were standardised by age and sex to give a T-score for each dimension. SCL-90-R dimension T-scores greater than 62 were used to indicate psychological morbidity as described in the SCL-90-R handbook (Degrotis, 1975). Psychological syndrome T-score profiles were allocated according to the SCL-90-R handbook. SCL-90-R dimension profile patterns were created using the K-means cluster analysis method. This allowed assessment of the psychological dimension profiles and their distribution between the RDC/TMD type 1a patients and control groups.

2.3.1.d. Pain/Symptom Assessment.

Pain was defined to be of muscle origin if palpation of jaw, head, neck and shoulder muscles (Helikimo 1974; Wolfe et al, 1990) in the sites reported to be painful were positive. Patients reporting pain without associated muscle tenderness were excluded. Patients were chosen to comply with the group 1 (Muscle disorders), category 1a (myofascial pain) of the orofacial research diagnostic criteria (Le Resche et al, 1992). After patient selection, pain and somatic symptom severity were retrospectively assessed using the scalar responses to the VAS and 13 questions from the SCL-90-R (Q1-headaches, Q4-faintness and dizziness, Q12-chest pain, Q14-low in energy or run down, Q27-low back pain, Q39-heart palpitations, Q40-nausea, Q42-muscle soreness, Q52-numbness or tingling, Q55-trouble concentrating, Q56-weakness, Q58-heavy feelings in limbs, Q66-restless or disturbed sleep) (Degrotis, 1975). An index of symptom prevalence was calculated as the total number of symptoms reported from the 54 symptom questions in the pain questionnaire. The SCL-90-R global indices were also used to assess inter-group differences. The SCL-90-R Global Symptom Index (GSI) is a measure of psychological distress combining symptom number and severity (Degrotis, 1975). The SCL-90-R Positive Symptom Distress Index (PSDI) is a measure of psychological severity corrected for symptom number, whilst the Positive Symptom Total (PST) is a measure of psychological symptom prevalence (Degrotis, 1975). Patients were instructed to report any body symptoms, irrespective of their impression of the origin, and not to restrict reporting to the orofacial region. This was done to avoid restricted reporting of whole body symptoms and to exclude the bias toward patients reporting only orofacial symptoms that may be perceived to be of importance in assessment of their orofacial condition.

2.3.2. CFS Group.

CFS patients and, age and sex matched controls subjects were recruited over an 18-month period, September 1994 - February 1996. The statistician from the Royal North Shore Hospital calculated the study group size to be a minimum of 80 subjects per arm. The CFS patients were recruited from sequential medical practitioner referrals to the Royal North Shore Hospital CFS clinical group from within the Sydney metropolitan area and advertisement in an ME/CFS newsletter. Two hundred and ten potential CFS subjects expressed an interest in participating in the study, of whom 114 agreed to participate and satisfied the defined diagnostic criteria (Sharpe et al, 1991). CFS patients were excluded from the study at any time, if an alternative diagnosis was made or if they did not comply with additional study requirements.

The age- and sex-matched control subjects were recruited from two sources; friends and relatives of the CFS patients, and from advertisement of the study at the University of Sydney and the University of Technology (Sydney). These two groups were selected to determine if there were any differences between control subjects with exposure to CFS patients and control subjects with no exposure to CFS patients. One hundred and eight control subjects were assessed and excluded if they reported a history of fatigue that influenced their lives or evidence of illness or psychiatric disorder. Eighty-six control subjects were recruited.

All study participants were requested to cease the use of any herbal, naturopathic or "alternative" medicine or vitamin supplements for one week prior to sample collection. On the day of clinical examination and sample collection subjects were clinically assessed by either of two endocrinologists (Phillip Clifton-Bligh, Greg Fulcher), completed specifically designed questionnaires, the John Hopkins Symptom Checklist-90-Revised (SCL-90-R) (Degrotis, 1975) and blood, microbiological swabs of nose and throat, venous blood and urine samples were collected. All subjects completed consent forms (Appendix 2). The specifically designed questionnaires included medical history, symptom prevalence and severity, onset and duration of illness, treatments used, social demographics and other factors which may influence their illness (Appendix 3). Within these questionnaires scalar responses to 12-month frequency and severity, and 7 day severity to facial pain and TMJ pain assessed the TMD symptoms. The clinicians questioned the patients of the presence or absence of facial pain. The questionnaire assessed the scalar severity response to facial and TMJ pain within the last 7 days and the last 12 months as well as a scalar response of the frequency of facial or TMJ pain over the last 12 months. No facial muscle or TMJ palpation was performed.

2.3.3. Statistical Analysis.

Data distributions were evaluated for violations of assumptions with parametric statistical analyses by assessment of normal distribution of each variable. All percentage data were arcsine transformed before analysis and those data not having a normal distribution were log transformed for usage in logistic regression analysis. Subject characteristics and symptom incidence was assessed using chi-square probability and students t-tests. Correction for multiplicity occurred where necessary. Sensitivity (Sen), defined as the prevalence of true positives in the RDC/TMD type 1a patient group, and specificity (Spec), defined as the percentage of true negatives in the control group, was determined. Univariate group differences were assessed on untransformed data using the non-parametric Mann Whitney U test. Group differences were further assessed using multivariate standard and forward-stepwise discriminate function analyses on the transformed data (significance $p \leq 0.05$). These data were processed using Access97 ™ (Microsoft, Redmond, WA, USA), Excel97 ™ (Microsoft) and Statistica ™ (Ver. 5.1, Statsoft, Tulsa, OK, USA).

2.4. RESULTS.

2.4.1. RDC/TMD Type 1a patients.

2.4.1.a. Patient Characteristics.

Of the 46 RDC/TMD type 1a patients interviewed, who met the criteria, three were excluded because of incomplete questionnaires. One of the 41 control subjects was excluded because of a positive response to the VAS and a history of chronic pain within the previous 12 months. There were no differences between the age and sex characteristics of the RDC/TMD type 1a patients and the control (C) groups (RDC/TMD type 1a: mean age 39.5±11.6 (SD) years, range 16-62 years, 79.1% female; C: mean age 35.5±15.2 years, range 11-72 years, 70% female). No difference was found in the number of patients or subjects in long-term marital or de-facto relationships (29 and 25, RDC/TMD type 1a patients and C respectively). No statistically significant difference was found in the ethnic backgrounds of the study participants. All except two were born in Australia (both of Chinese origin). The majority of study subjects were of Western European ethnic origin (Anglo-Celtic-Germanic - 74/83, 89.2%), seven (7/83, 8.4%) of Eastern European origin (Croatian / Slavic) and two of oriental origin (Chinese, 2/83, 2.4%).

2.4.1.b. Pain Distribution.

Although all RDC/TMD type 1a patients reported a history of orofacial muscle pain, on the day of the clinical examination they reported pain: 1) 38 in neck, shoulder and thoracic spine (88%); 2)35 in face and head (81%); 3) 30 in lower limb (70%); 4) 29 in low back (63%); 5) 24 in upper limb (56%); 6) 17 in abdomen (40%); and 7) 14 in anterior chest wall and sternum (33%). It was also found that three patients (7%) presented with pain restricted to the head, 12 (28%) with regional pain distributed predominantly in a facio-scapulo-humeral distribution, and 28 (65%) had pain in all four quadrants. Thus 7.0% were classified as having orofacial pain alone, 28% were classified as having myofascial pain syndrome, and although the fibromyalgia points were not palpated, 65% had a pain distribution consistent with fibromyalgia. Interestingly, limb and anterior chest wall and/or sternal pain were only reported by patients with pain in all four quadrants, i.e. the fibromyalgia-like patients.

At onset, head pain was the first site of pain reported by 20 of the 43 RDC/TMD type 1a patients (47%), with 30 (70%) reporting a facio-scapulo-humeral distribution. However, eight (19%) also reported pain to be first noticed in the low back. Most patients reported localised or regional pain at onset, which progressed too more generalised pain, involving all four quadrants in 28 (65%) patients in this cohort.

2.4.1.c. Pain Assessment.

As previously described there was an increase in the VAS (Mean±SEM RDC/TMD type 1a patients = 2.72±0.76; C = 0) between the RDC/TMD type 1a patients and C groups. Forty-two of 43 (98%) RDC/TMD type 1a patients had a VAS score >2. Forward stepwise discriminate function analysis using all pain symptom indicators and the SCL-90-R global indices, excluding the VAS, was used to assess inter-group differences. Table 2.2 shows that symptom prevalence, SCL-90-R questions 42 (muscle soreness), 14 (low energy or run down - lethargy/fatigue) and 40 (nausea), were important factors in differentiating between the RDC/TMD type 1a patient and control groups. The SCL-90-R global indices did not appear to be important inter-group determinants and as a result, the RDC/TMD type 1a patients in this cohort, selected on the basis of muscle pain, also had increased symptom prevalence, fatigue and nausea compared with control subjects.

Table 2.2. Forward stepwise regression analysis of pain assessment questions found important in determining the difference between the RDC/TMD type 1(a) patients and control subjects.

Pain/symptom indicators	Wilks' λ	F (1,75)	P
Symptom prevalence	0.443	7.09	<0.009
Q42 Muscle soreness	0.462	10.59	<0.002
Q14 Low in energy or slowed down	0.429	4.46	<0.04
Q40 Nausea	0.438	6.18	<0.02
Q27 Low back pain	0.423	3.46	0.066
Q39 Palpitations	0.422	3.23	0.076
Q58 Heaviness in limbs	0.417	2.31	0.132

Model: Wilks' $\lambda = 0.405$; $P<0.00001$

When the patients with four quadrant pain (fibromyalgia-like group, n=28) were compared with the remainder of the RDC/TMD type 1a patients (Other group, n=15), forward stepwise discriminate function analysis revealed that SCL-90-R questions 40 (nausea, $P<0.003$), 14 (low energy or run down - lethargy/fatigue, $P<0.002$), 42 (muscle soreness), and symptom prevalence, were also important factors in differentiating between the fibromyalgia-like and the other group patients (Model: Wilks' λ: 0.387, F=15.03, $P<0.00001$).

2.4.1.d. Signs and Symptoms.

Of the 54 symptoms reported in the CPRU questionnaire, Table 2.3 lists the sensitivity and specificity of the 35 symptoms with increased prevalence in the RDC/TMD type 1a patients (multiplicity correction $P\leq0.005$). The symptoms could be grouped into musculoskeletal, gastrointestinal, genitourinary, neurological and infection-related groups. There were highly significant prevalence differences in the musculoskeletal, gastro-genitourinary and infection-related symptoms between the RDC/TMD type 1a patient and C groups. The fibromyalgia-like group also reported increased symptom prevalence (Fibromyalgia-like = 21.2±10.6 v Other = 13.1±8.5, $P<0.01$), in particular nausea (Sen. = 88%, Spec = 78%; $P<0.001$) and dizziness (Sen. = 72%, Spec = 83%; $P<0.001$) when compared with the other MP patients (Multiplicity correction - $P\leq0.005$).

Table 2.3. Sensitivity and specificity of questionnaire reported symptoms in RDC/TMD type 1(a) patients and control subjects.

Symptom	Sensitivity (%)	Specificity (%)	*P*
Musculoskeletal symptoms			
Headaches	88.4	55.0	<0.001
Tension headaches	69.8	70.0	<0.001
TMJ clicking	67.4	82.5	<0.001
Muscle weakness	65.1	100.0	<0.001
Morning joint stiffness	62.8	85.0	<0.001
Muscle fatigue	60.5	92.5	<0.001
Muscle twitches	46.5	92.5	<0.001
Muscle cramps	41.9	95.0	<0.001
Parafunction	39.5	90.0	<0.005
Sciatica	32.6	100.0	<0.001
Gastrointestinal symptoms			
Nausea	65.1	92.5	<0.001
Abdominal bloating	51.2	87.5	<0.001
Abdominal pain	48.8	97.5	<0.001
Irritable bowel syndrome	44.2	95.0	<0.001
Low grade diarrhoea	41.9	92.5	<0.001
Gastric reflux	39.5	95.0	<0.001
Constipation	32.6	100.0	<0.001
Genitourinary symptoms			
Urinary frequency	55.8	90.0	<0.001
Groin lymphodynia	39.5	97.5	<0.001
Recurrent genital infections	25.5	100.0	<0.001
Dysuria	23.2	97.5	<0.005
Menstrual pain (females)	64.7	72.0	<0.005
Infection related symptoms			
Fatigue or lethargy	65.1	95.0	<0.001
Low grade fever	48.8	95.0	<0.001
Cervical lymphodynia	55.8	92.5	<0.001
Axial lymphodynia	44.2	92.5	<0.001
Recurrent sore throats	44.2	90.0	<0.001
Neurological symptoms			
Hyperaesthesia	51.2	90.0	<0.001
Paraesthesia	41.9	97.5	<0.001
Other symptoms			
Cardiac dysrhythmias	46.5	95.0	<0.001
Chest pain	32.6	95.0	<0.005
Allergies	53.5	87.5	<0.001
Dizziness of fainting spells	48.8	95.0	<0.001
Tinnitus	44.2	90.0	<0.001
Earaches	32.6	92.5	<0.001

Statistical methods = Chi-square Probability tests. Multiplicity corrected p-value - $p \leq 0.005$.

2.4.1.e. Onset-related Events.

Thirty-one of the 43 (71.2%) associated onset of their symptoms with one or more causally-related events including upper respiratory influenza-like infection, low-grade diarrhoea, genitourinary infection, glandular fever or trauma (Table 2.4). Pain and symptom onset was reported to be either sudden (13/43 - 30.2%) or gradual (30/43 - 69.8%). A stressful event at onset was reported by nine of the 43 (20.9%). Patients with sudden pain/symptom onset were more likely to report an infective event associated with onset: upper respiratory / influenza like infections (Sudden=11/13 v Gradual=5/30, P<0.001) or genitourinary tract infections (Sudden=3/13 v Gradual=1/30, P<0.05). Interestingly, pain onset following a new sexual contact was reported by 3 of 43 (7%) RDC/TMD type 1a patients all of whom reported a genitourinary infection at onset, one of who also reported glandular fever at onset. Gradual pain / symptom onset patients were more likely to be unable to identify an onset associated event (Sudden=0/13 v Gradual=12/30, P<0.007). Interestingly, prescription of antibiotics at onset was reported by 13 patients and occurred more frequently in the sudden onset patients than gradual onset patients (Sudden 7/13 v 5/30 - P<0.007).

Table 2.4. Prevalence of major onset-related events in RDC/TMD type 1a patients.

Onset Event	Sudden Onset	Gradual Onset	Total Prevalence
Upper respiratory / Influenza-like infection	11/13 (84.6%)	5/30 (16.7%)	16/43 (37.2%)
Diarrhoea	4/13 (30.7%)	6/30 (20.0%)	10/43 (23.3%)
Trauma	2/13 (15.7%)	3/30 (10.0%)	5/43 (11.6%)
Genitourinary Infection	3/13 (23.0%)	1/30 (3.3%)	4/43 (9.3%)
Glandular Fever	1/13 (7.7%)	3/30 (10.0%)	4/43 (9.3%)
No associated event	0/13 (0%)	12/30 (40.0%)	12/43 (27.9%)

2.4.1.f. History of Infectious Events.

RDC/TMD type 1a patients had increases in: 1) history of chronic recurrent upper respiratory tract infection (Sen=39.5%, Spec=95%; P<0.001); 2) history of genitourinary infection (Sen=37.2%, Spec=82.4%; P<0.05); and 3) history of appendectomies (Sen=23.2%, Spec=92.5%; P<0.05). No differences were noted for reporting of glandular fever.

2.4.1.g. Pain in Long-term Partners.

In the RDC/TMD type 1a patient group, a total of 29 patients (67%) were in long-term relationships, of whom 17 (Sen=39.5%, Spec=90%; P<0.02) reported that their partner had chronic muscle pain of varying severity and distribution. When the study subjects in long-term relationships were compared between the RDC/TMD type 1a patient and C groups, the increase was even more pronounced (Sen=58.6%, Spec=84%; P<0.002). Both of the control subjects who reported that their partners had muscle pain, also responded positively to at least one of the SCL-90-R pain/symptom indicator questions.

2.4.1.h. SCL-90-R Group Differences.

Table 2.5 shows the analysis of the responses to the various SCL-90-R global indices and dimension scores. The major differences between the groups were the increased global indices and somatization score with higher scores also noted in the obsessive compulsive and depression. Somatization was the only group dimension in the SCL-90-R defined psychopathological range (>62) and there was an increased prevalence of somatization T-scores >62 in the RDC/TMD type 1a patients (25/43 v 6/40, $P<0.0001$). No other dimension T-score >62 was found to have an increased prevalence when RDC/TMD type 1a patient and C groups were compared.

Table 2.5. Mean SCL-90-R global and dimension t-scores in RDC/TMD type 1(a) patients and control subjects.

SCL-90-R PARAMETER	RDC/TMD type1(a)	Control	*P*
GLOBAL INDICIES	**(X±SE)**	**(X±SE)**	
Global Severity Index (GSI)	58.0±1.32	48.8±1.78	<0.02
Positive Symptom Total (PST)	58.1±1.17	50.7±1.77	<0.05
Positive Symptom Distress (PSDI)	60.5±0.97	52.3±1.15	<0.0008
DIMENSION SCORES			
1. Somatization	64.2±1.15	50.2±1.73	<0.000001
2.Obsessive-Compulsive	58.1±1.42	49.8±1.69	<0.02
3.Interpersonal Sensitivity	51.9±1.59	52.9±1.60	NS
4.Depression	58.0±1.35	49.9±1.77	<0.03
5.Anxiety	52.3±1.56	48.8±1.53	NS
6.Hostility	50.5±1.27	49.8±1.43	NS
7.Phobic Anxiety	49.6±1.22	47.8±1.07	NS
8.Paranoid Ideation	48.2±1.37	49.1±1.47	NS
9.Psychoticism	54.8±1.32	50.8±1.36	NS

Statistical methods = Mann Whitney U test ($p \leq 0.05$).

The fibromyalgia-like patients had increased SCL-90-R global indices for the global severity index (GSI) ($P<0.005$) and positive symptom total (PST) ($P<0.02$), but not positive symptom distress index (PSDI) ($P<0.054$) when compared with the other muscle pain patients (Mann-Whitney U-test). Similarly, the fibromyalgia-like group had increased scores in the somatization ($P<0.0003$), depression ($P<0.004$), obsessive compulsion ($P<0.008$) and the anxiety dimensions ($P<0.02$), when compared with the other muscle pain patients. Discriminate function analysis of the difference between the fibromyalgia-like patients and other muscle pain, revealed that somatization ($P<0.03$) was the prime assessment difference between the two groups (Model: Wilks' Lambda=0.53, F=6.56, $P<0.0002$) with the hostility ($P<0.05$) and the PST index ($P<0.005$) being the 2nd and 3rd variables, respectively. Depression and anxiety were not important discriminate dimensions in this model.

2.4.1.i. SCL-90-R Global Index Associations with Dimension and Pain/Symptom Parameters.

Regression analysis of the GSI against the dimension T-scores revealed important associations, in order of discriminate ability, with the depression ($P<0.000000001$), obsessive compulsion ($P<0.0000004$), somatization ($P<0.00000006$), interpersonal sensitivity dimensions ($P<0.00008$) and a negative association with the phobic anxiety dimension ($P<0.03$) (Model: $R^2=0.960$, $F=255.87$, $P<0.00001$). The GSI was also associated with SCL-90-R Q66 (restless or disturbed sleep, $P<0.15$), Q52 (numbness and tingling, $P<0.008$) and Q12 (chest pain, $P<0.007$) (Model: $R^2=0.413$, $F=13.71$, $P<0.000001$). The GSI was not associated with the index of symptom prevalence or the VAS and was not an important factor in determining the difference between the RDC/TMD type 1a patient and control groups.

Regression analysis of the PSDI against the dimension T-scores revealed a significant association only with the somatization dimension ($P<0.0000002$; Model: $R^2=0.529$, $F=9.10$, $P<0.00001$). The PSDI was also associated with SCL-90-R Q1 (headaches, $P<0.002$), Q42 (muscle soreness, $P<0.002$) and Q52 (numbness and tingling, $P<0.002$) (Model: $R^2=0.542$, $F=5.29$, $P<0.000001$). The PSDI was not associated with the index of symptom prevalence or the VAS or important in determining differences between the RDC/TMD type 1a patient and control groups.

Regression analysis of the PST against the dimension T-scores revealed strong associations, in order of discriminate ability, with depression ($P<0.000002$), interpersonal sensitivity ($P<0.007$) and obsessive compulsion dimensions ($P<0.004$) (Model: $R^2=0.887$, $F=99.92$, $P<0.00001$). The PST was also associated with SCL-90-R Q66 (Restless or disturbed sleep, $P<0.06$), Q52 (numbness and tingling, $P<0.07$) and Q12 (chest pain, $P<0.21$) (Model: $R^2=0.325$, $F=9.39$, $P<0.000001$) and not with symptom prevalence or VAS.

2.4.1.j. Pain/symptom indicators and SCL-90-R Dimension Differences.

Five pain/symptom indicators, VAS, index of symptom prevalence, SCL-90-R questions 14 (Low energy/ lethargy / fatigue), 40 (nausea) and 42 (muscle soreness), were associated with differences between the RDC/TMD type 1a patient and C groups. Analysis of the dimension score changes with these pain/symptom indicators was undertaken to assess the association between each dimension and pain expression.

Regression analysis of the VAS against the dimension T-scores revealed that important changes in dimension scores occurred with increasing VAS scores (Model: $R^2=0.337$, $F=4.12$,

$P<0.0003$). A positive relationship was found between the VAS and somatization ($P<0.0002$) and a negative relationship with interpersonal sensitivity ($P<0.02$). The index of symptom prevalence was associated with strong dimensional changes (Model: $R^2=0.614$, $F=4.91$, $P<0.00004$). A positive relationship was found between the index of symptom prevalence and somatization ($P<0.008$), obsessive compulsion ($P<0.05$) and psychoticism ($P<0.05$), and a negative relationship with interpersonal sensitivity ($P<0.04$). SCL-90-R question 42 (muscle soreness) was associated with pronounced dimensional changes (Model: $R^2=0.439$, $F=6.36$, $P<0.00001$) and had a positive relationship with the somatization dimension ($P<0.000002$). SCL-90-R question 14 was also associated with significant dimensional changes (Model: $R^2=0.372$, $F=4.81$, $P<0.00005$) and had a negative relationship with interpersonal sensitivity ($P<0.005$). No relationship was found between Q14 and the depression dimension although Q14 forms part of that dimension. SCL-90-R question 40 (nausea) was also associated with strong dimensional changes (Model: $R^2=0.392$, $F=5.23$, $P<0.00002$) and had a positive relationship with somatization ($P<0.02$) and a negative relationship with interpersonal sensitivity ($P<0.04$). No association was found between any of the pain/symptom indicators and the depression and anxiety dimension scores.

Three dimensions had increased responses in the RDC/TMD type 1a patients; somatization, obsessive compulsion and depression. Logistic regression analysis of the somatization dimension against the pain/symptom indicators found positive associations with SCL-90-R questions 52 (numbness and tingling; $P<0.009$) and Q42 (muscle soreness; $P<0.04$) (Model: $R^2=0.592$, $F=6.49$, $P<0.000001$). The obsessive compulsion dimension was positively associated with SCL-90-R question Q55 (trouble concentrating, $P<0.008$), Q52 (numbness and tingling; $P<0.009$) and Q12 (pain in heart or chest, $P<0.04$). The depression dimension was not associated with any of the pain/symptom indicators. The anxiety dimension was positively associated with SCL-90-R question 39 (heart pounding or racing, $P<0.05$) and negatively associated with VAS assessment scores ($P<0.05$) (Model: $R^2=0.452$, $F=3.68$, $P<0.0002$).

2.4.1.k. SCL-90-R Question Response Interpretation.

The SCL-90-R is a symptom checklist and includes questions that are subject to different clinical interpretations (Table 2.6). There were no associations between the VAS, symptom prevalence or muscle soreness and the SCL-90-R symptom scores, whilst the SCL-

90-R symptoms were increased in the fibromyalgia-like patients compared with the remaining RDC/TMD type 1a patients.

Table 2.6. The clinical characteristics of the 29 elevated muscle pain-related SCL-90-R questions.

Symptom Division	SCL-90-R Interpretation
PHYSICAL SYMPTOMS	
1. Headaches	Somatization
4. Faintness or dizziness	Somatization
12. Pains in heart or chest	Somatization
27. Pains in lower back	Somatization
40. Nausea or upset stomach	Somatization
42. Muscle soreness	Somatization
48. Trouble getting your breath	Somatization
49. Hot or cold spells	Somatization
52. Numbness or tingling in parts of your body	Somatization
53. A lump in your throat	Somatization
56. Weak feelings in body	Somatization
58. Heavy feelings in the arms or legs	Somatization
17. Trembling	Anxiety
39. Heart pounding or racing	Anxiety
SLEEP DISTURBANCE	
44. Trouble falling asleep	Additional items
64. Awakening in the early morning	Additional items
66. Sleep that is restless or disturbed	Additional items
COGNITIVE EVENTS	
9. Trouble remembering things	Obsessive Compulsion
38. Having to do things very slowly to ensure correctness	Obsessive Compulsion.
46. Difficulty making decisions	Obsessive Compulsion.
51. Mind going blank	Obsessive Compulsion.
55. Trouble concentrating	Obsessive Compulsion.
POSSIBLE DISEASE CONSEQUENCES	
5. Loss of sexual interest or pleasure	Depression
14. Feeling low in energy or slowed down	Depression
71. Feeling everything is an effort	Depression
87. The idea that something serious is wrong with your body	Psychoticism
POSSIBLE PSYCHOLOGICAL RESPONSES	
30. Feeling blue	Depression
32. Feeling no interest in things	Depression
50. Having to avoid certain things, places or activities because they frighten you.	Phobic anxiety

Therefore the SCL-90-R responses were assessed from two aspects; the responses were interpreted as either: 1) a psychological illness with associated physical symptoms; or 2) a physical illness with psychological consequences. The prevalence of positive responses and increased scalar responses to each of the 90 questions within the SCL-90-R were determined for the RDC/TMD type 1a patient and control groups. Table 2.6 shows the two different interpretations for the twenty-nine questions that had different responses between the RDC/TMD type 1a patients and the control subjects.

The SCL-90-R interpretation shows differentiation of the somatization, obsessive compulsion and depression dimensions. The somatization dimension could be interpreted to indicate simple physical symptom reporting. The obsessive compulsion dimension responses could be interpreted to indicate cognitive changes. The sleep disturbance symptoms were classified as additional items by the SCL-90-R, whilst the questions from the depression dimension could be interpreted as reactive psychological responses, or symptoms or consequences of the disease condition. These data show that the SCL-90-R determined differentiation may be interpreted in a second manner that does not support the notion that TMD patients have an affective psychological disorder.

2.4.2. TMD in CFS patients.

One hundred and fourteen potential CFS patients were recruited (31 males; 83 females) and 14 were excluded (4 male; 10 female) following review of clinical, history and SCL-90-R data. Three of the excluded patients presented with hyperthyroidism, 1 with current acute Ross River virus infection, 2 with elevated antinuclear antibody (ANA) titres (>320), and the remaining 5 with a psychiatric diagnosis.

Eighty-six age- and sex-matched control subjects were recruited (24 males; 62 females) and three were excluded (one male; two female). Two of the excluded subjects had psychiatric diagnosis, one of whom also reported fatigue, whilst the third had cardiac disease.

There was no difference in any characteristics when comparing the 100 CFS patients (age = 29.1±12.5 years; range 13-73 years; female 73%) with the 83 control subjects (age = 32.8±13.9 years; range 14-69 years; female 72%).

2.4.2.a. CFS Patients with TMD Symptoms.

Facial pain was reported to the clinicians by 29 (29%) of the 100 CFS patients. The difference in the facial pain symptoms reported to the clinicians and the frequency and severity of facial muscle pain or discomfort and jaw joint pain or discomfort over the preceding 12 months reported in the questionnaire was assessed. No TMD muscle or joint pain symptoms were reported by 61 (81%) of controls compared with 27 (31%) of CFS patients ($P<0.0001$). Eight (11%) control subjects and 20 (23%) of CFS patients reported either facial or TM joint pain ($P<0.04$) alone and 6 (8%) control subjects and 40 (46%) CFS patients report both facial muscle and TM joint pain ($P<0.0001$). Table 2.7 shows the patient reported scalar responses for the frequency and the severity of symptoms over the preceding 12 months.

Table 2.7. The prevalence of the scalar frequency and severity responses by CFS patients and control subjects.

Response	Face Pain Control	Face Pain CFS	*P*	TMJ Pain Control	TMJ Pain CFS	*P*
Frequency	N(%)	N(%)		N(%)	N(%)	
Never	67(89.3)	45(51.1)	<0.0001	64(84.2)	30(34.5)	<0.0001
Rarely	3(4.0)	15(17.0)	<0.01	6(7.9)	20(23.0)	<0.02
Some of the time	4(5.3)	24(27.3)	<0.0003	3(3.9)	28(32.2)	<0.0001
Most of the time	1(1.3)	4(4.5)	NS	3(3.9)	8(9.2)	NS
All of the time	0	0	NS	0	1(1.1)	NS
Severity						
Not at all	68(91.9)	48(55.8)	<0.0001	65(86.7)	35(39.8)	<0.0001
A little bit	3(4.1)	16(18.6)	<0.007	4(5.3)	19(21.6)	<0.004
Moderately	2(2.7)	13(15.1)	<0.01	2(2.7)	22(25.0)	<0.0001
Quite a bit	1(1.4)	8(9.3)	<0.04	2(2.7)	10(11.4)	<0.04
Extremely	0	1(1.2)	NS	2(2.7)	2(2.3)	NS

Table 2.8 shows the cumulative scores for a) face and TMJ pain frequency, and b) face and TMJ pain severity, for the CFS patients and control subjects. The CFS patients who reported that they had face pain, had higher frequency and severity of face pain responses (Frequency = $P<0.0004$; Severity = $P<0.0002$) and frequency and severity of TMJ pain (Frequency = $P<0.02$; Severity = $P<0.02$). These data show an increase in frequency and severity of TMD symptoms in CFS patients compared with controls, and that those study subjects who reported TMD symptoms, also have an increased 12-month frequency and severity of TMD symptoms. CFS patients who reported TMD symptoms had a higher frequency of reporting of all symptoms compared with CFS patient who did not report TMD symptoms (CFS TMD = 18.2±4.0; CFS No TMD = 11.7±7.6 - $P<0.0002$). CFS patients who reported TMD signs in their questionnaires had a higher frequency of reporting of all symptoms compared with CFS patient who did not report TMD signs (CFS TMD = 17.4±3.8; CFS No TMD = 14.1±4.9 - $P<0.0004$). These data show that TMD symptom expression in CFS patients is the same as that noted in the normal population but that TMD symptoms do have an increased prevalence in CFS patients and are found in the patients with more severe symptoms.

Table 2.8. The prevalence of the combined scalar frequency and severity responses for the facial and TMJ pain per subject for the CFS patients and control subjects.

Response	Frequency Index Control	Frequency Index CFS	*P*	Severity Index Control	Severity Index CFS	*P*
Number of Responses	N=83 N(%)	N=100 N(%)		N=83 N(%)	N=100 N(%)	
0	61(81.3)	27(31.0)	<0.0001	63(85.1)	31(36.5)	<0.0001
1	5(6.7)	12(13.8)	NS	4(5.4)	9(10.6)	NS
2	3(4.0)	12(13.8)	<0.04	1(1.4)	15(17.6)	<0.001
3	3(4.0)	14(16.1)	<0.02	3(4.1)	13(15.3)	<0.03
4	1(1.3)	14(16.1)	<0.002	1(1.4)	7(8.2)	NS
5	1(1.3)	4(4.6)	NS	0	2(2.4)	NS
6	1(1.3)	3(3.4)	NS	2(2.7)	6(7.1)	NS
7	0	1(1.1)	NS	0	1(1.2)	NS
8	0	0	-	0	1(1.2)	NS

Figure 2.1 shows the 12-month cumulative totals of frequency, severity, and TMD complexity, as well as the prevalence of subjects reporting clinical symptoms, face pain and TMJ pain, in association with age. This shows the distribution of TMD disorders with the highest levels in the 25-44 year age groups. There was an unexpected increase in subjects >55 years of age, which results from the disproportionately low control subject numbers in that age group compared with the number of CFS patients. The age of onset and severity of TMD symptoms are the same in CFS patients as with the RDC/TMD type1a patients.

2.4.2.b. Symptoms associated with 12-month Facial Pain Symptom Frequency in CFS patients.

Scalar frequency response (0-4) to the reporting of facial muscle pain compared with 12 month frequency responses (127 questions), was assessed by forward stepwise multiple regression analysis to determine which symptom responses also increased in frequency. There was a strong regression model (R^2=0.798, F=35.150, *P*<0.00000) with TMJ pain (+ve, $P<1.0E^{-11}$) being the primary symptom association, followed by Q64 (awakening in the early morning +ve, *P*<0.00006) and Q111 (hair loss +ve, *P*<0.0000003). Table 2.9 shows the symptoms that had strong correlations (multiplicity correction - *P*<0.005) with the scalar frequency response for facial muscle pain for both the CFS patients and control subjects. Two symptoms correlated in both control subjects and CFS patients, six additional symptoms were correlated with the frequency of facial pain in the control subjects and 24 additional symptoms were correlated with the frequency of facial pain in CFS patients. In the control subjects seven symptoms were physical symptoms and one was a psychological response, whilst in the CFS patients 18 were physical symptoms and eight were psychological responses. In both CFS patients and control subjects, increasing frequency of facial pain was associated with both physical symptoms and psychological response differences.

The CFS patients with face pain had an increased prevalence of 5 symptoms reported to clinicians: 1) joint pain (FP=77%; NoFP=51% - $P<0.006$); 2) pain (FP=59%; NoFP=34% - $P<0.02$); 3) fever (FP=55%; NoFP=32% - $P<0.02$); 4) sore throat (FP=77%; NoFP=57% - $P<0.04$); and 5) cervical lymphodynia (FP=66%; NoFP=45% - $P<0.04$). These data show that CFS patients with face pain had an increased prevalence of reporting of infection-like symptoms compared with CFS patients who do not have face pain.

Figure 2.1. Plot of different measures of TMD symptom expression in relationship to the age of the study subjects.

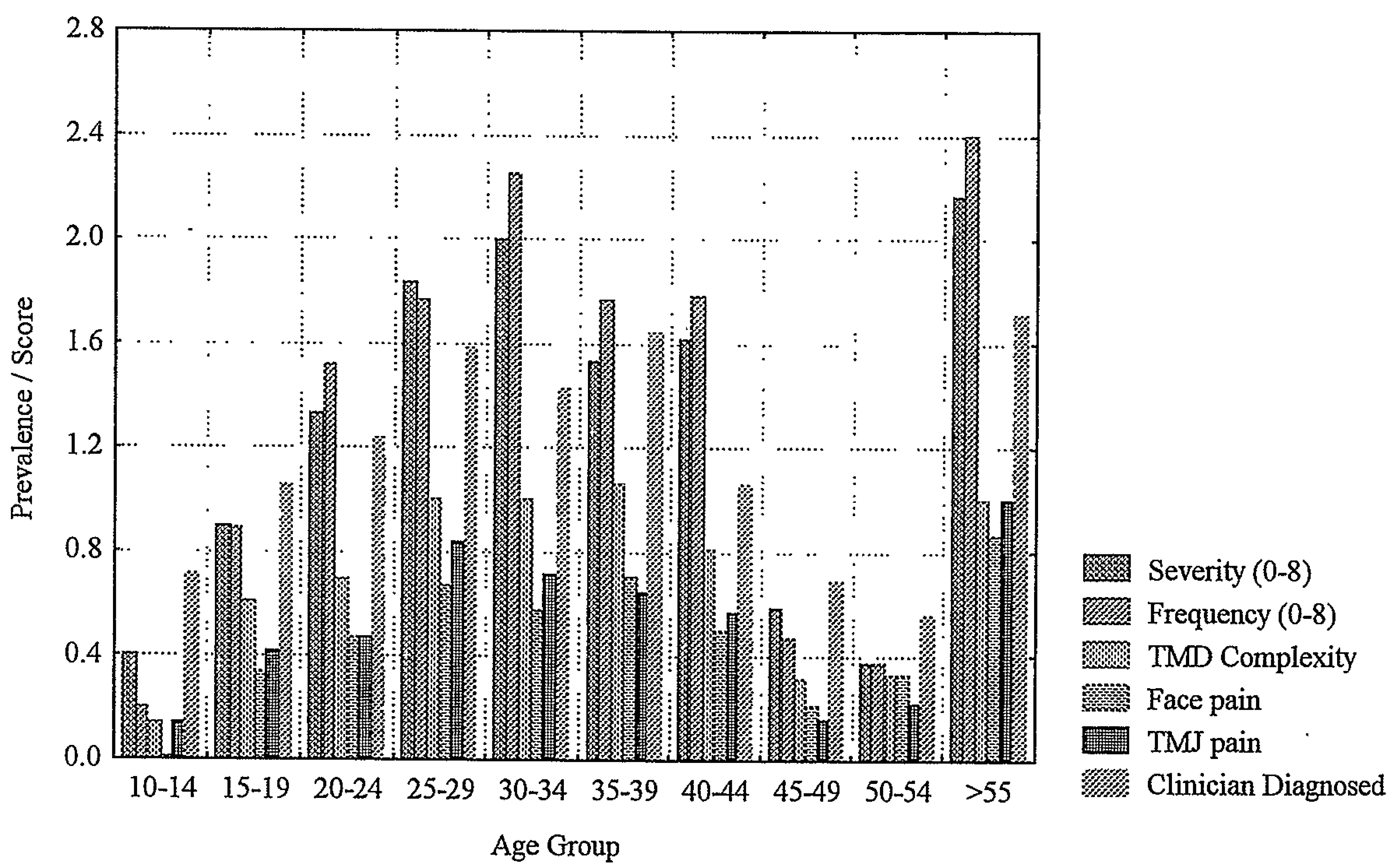

Table 2.9. Summary of the Spearman Rank correlation analysis of the scalar responses to the 12-Month frequency and severity scores of the scalar responses for facial pain for the CFS patients and the control subjects.

Question	Frequency Control	Severity Control	Frequency CFS	Severity CFS
Physical Symptoms	r P	r P	r P	r P
Q105 TMJ pain or discomfort	0.59 <0.001	0.51 <0.001	0.67 <0.001	0.64 <0.001
Q122 Earaches	0.34 <0.004	0.44 <0.001	0.42 <0.001	0.32 <0.003
Q125 Tired eyes	0.31	0.39 <0.001	0.36 <0.001	0.35 <0.001
Q42 Muscle soreness	0.25	0.37 <0.002	0.39 <0.001	0.39 <0.001
Q121 Stomach cramps	0.39 <0.001	0.38 <0.001	0.29	0.29
Q95 Diarrhoea	0.39 <0.001	0.40 <0.001	0.29	0.29
Q115 Muscle twitches	0.30	0.40 <0.001	0.30 <0.005	0.27
Q104 Dry eyes	0.32	0.17	0.45 <0.001	0.43 <0.001
Q124 Swallowing difficulties	0.31	0.05	0.42 <0.001	0.39 <0.001
Q66 Restless/disturbed sleep	0.26	0.30	0.50 <0.001	0.36 <0.001
Q113 Joint pain	0.05	0.15	0.50 <0.001	0.46 <0.001
Q108 Feverishness/night sweats	0.26	0.17	0.44 <0.001	0.39 <0.001
Q109 Frequent urination	0.10	0.23	0.41 <0.001	0.31 <0.004
Q92 Allergies	0.05	-0.09	0.35 <0.001	0.32 <0.003
Q102 Ringing in the ears	0.25	0.20	0.32 <0.003	0.34 <0.002
Q51 Mind going blank	0.26	0.13	0.32 <0.003	0.33 <0.003
Q91 Abdominal pain	0.45 <0.001	0.28	0.25	0.28
Q127 Sensitive/bluish finger and toes	0.41 <0.001	0.20	0.10	0.30
Q4 Faintness and/or dizziness	0.36 <0.002	0.11	0.03	0.29
Q44 Trouble falling asleep	0.22	0.34 <0.004	0.18	0.20
Q64 Awakening early in the morning	0.09	0.26	0.40 <0.001	0.14
Q99 Burning urination (Dysuria)	0.09	0.13	0.38 <0.001	0.14
Q40 Nausea	0.18	0.16	0.36 <0.001	0.16
Q49 Hot or cold spells	0.21	0.09	0.32 <0.003	0.26
Q126 Tooth grinding	0.27	0.01	0.16	0.36 <0.001
Q58 Heavy feelings in arms or legs	0.22	0.30	0.26	0.35 <0.001
Q1 Headaches	0.08	0.09	0.20	0.35 <0.002
Q119 Rashes	0.07	-0.02	0.26	0.34 <0.002
Q46 Difficulty making decisions	0.12	0.15	0.25	0.33 <0.003
Q100 Confusion	0.17	0.25	0.23	0.32 <0.003
Psychological Responses				
Q90 Something seriously wrong with mind	0.05	-0.12	0.41 <0.001	0.31 <0.004
Q116 Nightmares	0.20	0.30	0.39 <0.001	0.36 <0.001
Q72 Spells of terror or panic	0.23	0.29	0.36 <0.001	0.33 <0.002
Q57 Feeling tense or keyed up	0.25	0.15	0.33 <0.002	0.30 <0.005
Q2 Nervousness or shakiness inside	0.33 <0.005	0.30	0.12	0.18
Q86 Thoughts/images of frightening nature	0.32	0.40 <0.001	0.25	0.22
Q80 Feeling something bad will happen	0.21	0.14	0.37 <0.001	0.29
Q89 Feelings of guilt	0.03	0.04	0.33 <0.002	0.26
Q31 Worrying to much about things	-0.04	0.07	0.32 <0.003	0.24
Q59 Thoughts of death/dying	0.06	0.04	0.32 <0.003	0.21
Q78 So restless can't sit still	0.25	0.14	0.29	0.31 <0.004

Multiplicity α correction ($P<0.005$).

2.4.2.c. 12-Month Facial Pain Symptom Severity in CFS Patients.

The scalar severity response (0-4) to the reporting of facial muscle pain against the 12-month severity responses was assessed by forward stepwise multiple regression analysis to determine which symptom responses also increased in severity. There was also a strong regression model (R^2=0.760, F=28.236, P<0.00000), with TMJ pain (+ve, P<2.0E^{-9}) being the primary symptom association followed by Q64 (awakening in the early morning +ve, P<0.0002) and Q92 (allergies +ve, P<0.0003). Table 2.9 shows the symptoms that had strong correlations (multiplicity correction - P<0.005) with the scalar severity response for facial muscle pain for both the CFS patients and the control subjects. Four symptoms correlated in the control subjects and the CFS patients, four additional symptoms were correlated with the severity of facial pain in the control subjects and 20 additional symptoms were correlated with the severity of facial pain in the CFS patients. In the control subjects eight symptoms were physical symptoms and one was a psychological response, in the CFS patients 19 were physical symptoms and five were psychological responses.

Increased severity of facial pain in the control subjects was associated with increased severity of different symptoms in the CFS patients. In both CFS patients and control subjects increasing severity of facial pain was also associated with both physical symptoms and psychological response differences.

2.4.2.d. 12-Month TMJ Pain Symptom Frequency in CFS Patients.

The scalar frequency response (0-4) to the reporting of TMJ pain against the modified SCL-90-R frequency responses (127 questions) was assessed by forward stepwise multiple regression analysis to determine which symptom responses also increased in frequency. There was a strong regression model (R^2=0.763, F=28.577, P<0.00000) with facial pain (+ve, P<1.0E^{-7}) being the primary symptom association, followed by Q103 (sore glands +ve, P<0.09) and Q64 (awakening in the early morning +ve, P<0.00006). Table 2.10 (a, b) shows which symptoms had a strong correlation (multiplicity correction - P<0.005) with the scalar frequency response for TMJ pain, for both the CFS patients and the control subjects. Eleven symptoms correlated in both control subjects and CFS patients, seven additional symptoms were correlated with the frequency of facial pain in the control subjects and 13 additional symptoms were correlated with the frequency of facial pain in the CFS patients. In the control subjects eighteen symptoms were physical symptoms and five were psychological responses whilst in the CFS patients 19 were physical symptoms and six were psychological responses.

Table 2.10a. Spearman Rank correlations of the scalar responses to the 12-month TMJ pain frequency and severity scores for CFS patients and the control subjects.

Question	Frequency Control	Severity Control	Frequency CFS	Severity CFS
Physical Symptoms				
Q123 Facial muscle pain	0.59 <0.001	0.51 <0.001	0.67 <0.001	0.64 <0.001
Q120 Sore throat	0.36 <0.002	0.36 <0.002	0.40 <0.001	0.36 <0.001
Q66 Restless/disturbed sleep	0.42 <0.001	0.45 <0.001	0.34 <0.002	0.28
Q122 Earaches	0.33 <0.004	0.37 <0.001	0.34 <0.002	0.22
Q126 Tooth grinding	0.43 <0.001	0.38 <0.001	0.18	0.34 <0.002
Q40 Nausea	0.42 <0.001	0.32	0.39 <0.001	0.31 <0.004
Q108 Feverishness / Night sweats	0.36 <0.002	0.32	0.44 <0.001	0.40 <0.001
Q104 Dry eyes	0.37 <0.001	0.25	0.43 <0.001	0.51 <0.001
Q42 Muscle soreness	0.37 <0.001	0.27	0.37 <0.001	0.34 <0.002
Q124 Swallowing difficulties	0.35 <0.002	0.12	0.42 <0.001	0.34 <0.002
Q91 Abdominal pain	0.28	0.38 <0.001	0.31 <0.005	0.34 <0.002
Q102 Ringing in the ears	0.48 <0.001	0.55 <0.001	0.12	0.16
Q95 Diarrhoea	0.42 <0.001	0.35 <0.002	0.18	0.20
Q44 Trouble falling asleep	0.36 <0.002	0.41 <0.001	0.13	0.14
Q119 Rashes	0.36 <0.002	0.30	0.29	0.50 <0.001
Q114 Leg cramps	0.29	0.34 <0.003	0.31 <0.004	0.30
Q125 Tired eyes	0.24	0.37 <0.002	0.25	0.35 <0.001
Q27 Pains in low back	0.32	0.31	0.33 <0.003	0.35 <0.001
Q121 Stomach cramps	0.23	0.31	0.36 <0.001	0.47 <0.001
Q113 Joint pain	0.19	0.22	0.53 <0.001	0.46 <0.001
Q109 Frequent urination	0.26	0.20	0.42 <0.001	0.36 <0.001
Q103 Sore glands	0.27	0.21	0.37 <0.001	0.32 <0.003
Q101 Recurrent genital infections	0.10	0.15	0.34 <0.002	0.31 <0.004
Q112 Heartburn/Gastric reflux	0.13	0.11	0.33 <0.003	0.33 <0.003
Q4 Faintness or dizziness	0.36 <0.002	0.25	0.13	0.17
Q12 Chest pain	0.35 <0.002	0.25	0.12	0.21
Q56 Feeling weak in parts of your body	0.33 <0.004	0.05	0.09	0.19
Q48 Trouble getting breath	0.33 <0.004	0.30	0.17	0.16
Q100 Confusion	0.22	0.41 <0.001	0.16	0.20
Q14 Feeling low in energy or run down	0.22	0.40 <0.001	-0.10	0
Q99 Burning urination (Dysuria)	0.23	0.24	0.39 <0.001	0.28
Q1 Headaches	0.20	0.11	0.26	0.37 <0.001
Q92 Allergies	0.19	0.13	0.18	0.35 <0.001
Q46 Difficulty making decisions	0.13	0.27	0.27	0.34 <0.001
Q58 Heavy feelings in arms or legs	0.29	0.31	0.27	0.31 <0.003
Q118 Poor circulation /cold hands or feet	0.21	0.16	0.13	0.30 <0.005

(Multiplicity α correction - $P<0.005$).

Increased frequency of TMJ pain in the control subjects was associated with increased frequency of different symptoms in the CFS patients. In both CFS patients and control subjects increasing frequency of facial pain was also associated with both physical symptoms and psychological response differences. The increasing frequency of TMJ pain was associated with a higher number of symptoms than found for face pain.

2.4.2.e. 12-Month TMJ Pain Symptom Severity in CFS Patients.

The scalar severity response (0-4) to the reporting of facial muscle pain against the modified SCL-90-R severity responses, was assessed by forward stepwise multiple regression analysis to determine which symptom responses also increased in severity. There was also a strong regression model (R^2=0.776, F=30.805, P<0.00000) with facial pain (+ve, P<1.0E^{-9}) being the primary symptom association followed by stomach cramps (+ve, P<0.00003) and recurrent genital infections (+ve, P<0.00008). Table 2.10 (a, b) shows the symptoms that had strong correlations (multiplicity correction - P<0.005) with the scalar severity response for TMJ pain for both CFS patients and control subjects. Seven symptoms correlated in both the control subjects and the CFS patients, 16 additional symptoms were correlated with the severity of TMJ pain in the control subjects and 16 additional symptoms were correlated with the severity of TMJ pain in the CFS patients. In the control subjects eighteen symptoms were physical symptoms and ten were psychological responses, whilst in the CFS patients 19 were physical symptoms and three were psychological responses.

Table 2.10b. Summary of the Spearman Rank correlation analysis of the scalar responses to the 12-month TMJ pain frequency and severity scores for CFS patients and the control subjects.

Question Psychological Responses	Frequency Control	Severity Control	Frequency CFS	Severity CFS
Q116 Nightmares	0.34 <0.003	0.38 <0.001	0.36 <0.001	0.35 <0.001
Q57 Feeling tense or keyed up	0.34 <0.003	0.33 <0.005	0.32 <0.004	0.26
Q110 Frustration	0.32	0.34 <0.004	0.20	0.28
Q2 Nervousness or shakiness inside	0.41 <0.001	0.40 <0.001	0.07	0.23
Q87 Something seriously wrong with body	0.41 <0.001	0.52 <0.001	0.08	0.09
Q86 Thoughts/images of frightening nature	0.30	0.39 <0.001	0.09	0.09
Q78 So restless can't sit still	0.31	0.28	0.31 <0.004	0.21
Q82 Afraid will faint in public	0.16	0.20	0.31 <0.004	0.30 <0.005
Q34 Feelings easily hurt	-0.06	-0.05	0.30 <0.005	0.30
Q72 Spells of terror or panic	0.34 <0.003	0.17	0.22	0.27
Q67 Urges to smash or break things	0.23	0.40 <0.001	0.14	0.09
Q96 Anxiety	0.23	0.38 <0.001	0.22	0.22
Q11 Easily annoyed or irritated	0.21	0.37 <0.001	0.15	0.18
Q13 Afraid in open spaces / streets	0.18	0.34 <0.003	0.15	0.10
Q90 Something seriously wrong with mind	0.15	-0.01	0.34 <0.002	0.26
Q33 Feeling fearful	0.28	0.26	0.27	0.33 <0.002

(Multiplicity α correction - P<0.005).

Increased severity of TMJ pain in the control subjects was associated with increased frequency of different symptoms in the CFS patients. In both CFS patients and control subjects increasing severity of TMJ pain was also associated with both physical symptoms and differences in psychological responses. The increasing severity of TMJ pain was associated with increased severity of different symptoms than found for face pain.

2.4.2.h. 7-Day TMD Symptoms associations in the CFS and Control Groups.

Table 2.11 summarises the symptom correlation associations for the 7-day facial and TMJ pain severity responses in the CFS patients and the control subjects.

The scalar severity response (0-4) to the 7-day reporting of facial muscle pain against the 7-day severity responses was assessed by forward stepwise multiple regression analysis to determine which symptom responses also increased in severity. In the CFS group there was a strong regression model (R^2=0.961, F=32.931, P<0.00000), with Q83 (Feeling that people will take advantage of you if you let them; +ve, P<0.0003) being the primary symptom association followed by Q104 (Dry eyes; +ve, P<0.0002) and Q19 (Poor appetite; +ve, P<2.0E^{-10}). In the control group there was also a strong regression model (R^2=0.999, F=907.79, P<0.00000), with Q103 (Sore glands; +ve, P<0.0000001) being the primary symptom association followed by Q27 (Low back pain; -ve, P<0.00002) and Q39 (Heart racing or pounding; +ve, P<0.18). Table 2.11 shows the symptoms that had strong correlations (multiplicity correction - P<0.005) with the scalar severity response for facial muscle pain for both the CFS patients and the control subjects. Fourteen symptoms correlated in the control subjects and the CFS patients, 13 additional symptoms were correlated with the severity of facial pain in the control subjects and five additional symptoms were correlated with the severity of facial pain in the CFS patients. In the control subjects 20 symptoms were physical symptoms and seven were psychological responses, in the CFS patients 14 were physical symptoms and five were psychological responses. Increased 7-day severity of facial pain in both the CFS group and the control group were associated with similar symptom profiles.

The scalar severity response (0-4) to the 7-day reporting of TMJ pain against the 7-day severity responses was assessed by forward stepwise multiple regression analysis to determine which symptom responses also increased in severity. In the CFS group there was a strong regression model (R^2=0.977, F=56.206, P<0.00000), with Q93 (Constipation; +ve, P<1.0E^{-11}) being the primary symptom association followed by Q126 (Tooth grinding; +ve, P<1.0E^{-7}) and Q13 (Feeling afraid in open spaces or on the streets; +ve, P<1.0E^{-10}). In the control group there was also a strong regression model (R^2=0.998, F=641.71, P<0.00000), with Q114 (Leg cramps; +ve, P<1.0E^{-14}) being the primary symptom association followed by

Q98 (Blurry vision; +ve, $P<0.00007$) and Q61 (Feeling uneasy when people are watching or talking; +ve, $P<3.0E^{-12}$). Table 2.11 shows the scalar severity response for TMJ pain for both the CFS patients and the control subjects.

Table 2.11. Summary of the Spearman Rank correlation analysis of the scalar responses to the 7-day face pain and TMJ pain severity scores for CFS patients and the control subjects.

Question Psychological Responses	Facial Pain CFS	Facial Pain Control	TMJ Pain CFS	TMJ Pain Control
Q123 Facial pain or discomfort	1	1	0.36 <0.003	0.65 <0.001
Q105 Jaw joint pain	0.36 <0.003	0.65 <0.001	1	1
Q91 Abdominal pain / Bloating	0.44 <0.001	0.61 <0.001	0.58 <0.001	0.47 <0.001
Q96 Anxiety	0.43 <0.001	0.66 <0.001	0.47 <0.001	0.44 <0.002
Q103 Sore glands	0.43 <0.001	0.72 <0.001	0.36 <0.004	0.63 <0.001
Q106 Fatigue	0.43 <0.001	0.52 <0.001	0.49 <0.001	0.46 <0.001
Q121 Stomach cramps	0.43 <0.001	0.47 <0.001	0.50 <0.001	0.52 <0.001
Q100 Confusion	0.41 <0.001	0.76 <0.001	0.60 <0.001	0.55 <0.001
Q113 Joint pain	0.41 <0.001	0.60 <0.001	0.52 <0.001	0.57 <0.001
Q104 Dry eyes	0.41 <0.001	0.41 <0.004	0.37 <0.002	0.51 <0.001
Q125 Tired eyes	0.39 <0.001	0.63 <0.001	0.43 <0.001	0.51 <0.001
Q115 Muscle twitches	0.38 <0.002	0.50 <0.001	0.44 <0.001	0.48 <0.001
Q110 Frustration	0.35 <0.005	0.62 <0.001	0.46 <0.001	0.47 <0.001
Q109 Frequent urination	0.49 <0.001	0.45 <0.001	0.54 <0.001	-
Q122 Earaches	0.46 <0.001	0.69 <0.001	-	0.51 <0.001
Q118 Poor circulation / cold hands and feet	-	0.62 <0.001	0.41 <0.001	0.65 <0.001
Q98 Blurry vision	-	0.55 <0.001	0.37 <0.002	0.61 <0.001
Q124 Swallowing difficulties	-	0.54 <0.001	0.44 <0.001	0.69 <0.001
Q126 Tooth grinding	-	0.51 <0.001	0.60 <0.001	0.61 <0.001
Q83 Feeling that people will take advantage of you if you let them	0.42 <0.001	-	0.51 <0.001	-
Q93 Constipation	0.34 <0.005	-	0.53 <0.001	-
Q102 Ringing in the ears	-	0.72 <0.001	-	0.47 <0.001
Q120 Sore throat	-	0.61 <0.001	-	0.54 <0.001
Q114 Leg cramps	-	0.53 <0.001	-	0.47 <0.001
Q94 Depression	-	0.48 <0.001	-	0.40 <0.005
Q117 Paranoia	-	0.47 <0.001	-	0.44 <0.002
Q108 Feverishness / Night sweats	0.50 <0.001	-	-	-
Q79 Feeling of worthlessness	0.37 <0.003	-	-	-
Q92 Allergies	0.34 <0.005	-	-	-
Q101 Recurrent genital infections	-	0.40 <0.006	-	-
Q107 Fear of closed spaces	-	0.40 <0.005	-	-
Q111 Hair loss	-	0.40 <0.005	-	-
Q116 Nightmares	-	0.40 <0.005	-	-
Q112 Heartburn / Gastric reflux	-	-	0.57 <0.001	-
Q127 Fingers / toes bluish colour sensitive to touch	-	-	0.55 <0.001	-
Q119 Rashes	-	-	0.39 <0.001	-
Q22 Feelings of being trapped or caught	-	-	0.37 <0.004	-
Q50 Having to avoid certain things, places, or activities	-	-	0.37 <0.004	-

(Multiplicity α correction - $P<0.005$).

Sixteen symptoms correlated in the control subjects and the CFS patients, six additional symptoms were correlated with the severity of TMJ pain in the control subjects and eight additional symptoms were correlated with the severity of TMJ pain in the CFS patients. In the control subjects 17 symptoms were physical symptoms and five were psychological responses, in the CFS patients 18 were physical symptoms and six were psychological responses. Increased 7-day severity of TMJ pain in both the CFS group and the control group were associated with similar symptom profiles. There was a strong similarity in the symptom profiles for both facial and TMJ pain in the CFS and control groups.

2.4.2.i. Differences in Onset-related events associated with TMD in CFS patients.

The onset-associated events were compared in CFS who reported that they had either face pain or TMJ pain during the previous 12 months. Table 2.12 shows that of 11 events assessed that only the patients who reported face pain in the previous 12 months had any differences in prevalence of any onset associated event. Increases in the prescribing of antibiotics at onset and reactions to drugs taken around onset were the only events increased in the facial pain patients.

Table 2.12. Prevalence of onset associated events in CFS patients with TMD symptoms.

Onset Event	Doctor Reported Face Pain		Questionnaire Face Pain		Questionnaire TMJ Pain	
	Positive	Negative	Positive	Negative	Positive	Negative
Number	N=25	N=61	N=43	N=39	N=57	N=29
Viral Infection	19	43	31	31	42	20
Pesticide/Chemical exposure	3	0	3	0	3	0
Trauma	2	5	2	5	4	3
Drug reaction	0	6	**6***	0	5	1
Stress (work, family, personal)	11	18	17	12	20	9
Unknown	1	9	4	8	5	7
Antibiotics prescribed at onset	3	6	**8***	1	8	1
Reaction to vaccine	1	5	3	3	3	3
Gastrointestinal infection	1	0	1	0	1	0
Genetic predisposition	0	1	0	1	0	1
Contact with a CFS patient	1	1	1	1	1	1

Chi-Square/Fischer exact analysis. $\alpha = P<0.05$; *= $P<0.05$.

Multiple regression analysis was applied to assess if any onset factors were associated with TMD symptoms in the CFS group. Forward stepwise regression analysis found strong associations between infections, trauma and stress at onset with symptom presentation but face or TMJ pain were not associated with these onset events. Within the CFS group, stressful events at onset (Model: $R^2=0.960$, $F=32.729$, $P<0.00000$) were associated with Q76 (others do not you give credit for your achievements +ve $P<0.00000002$), Q85 (the idea that you should be punished for your sins +ve $P<0.0000001$), Q107 (fear of closed spaces -ve

$P<0.000000004$) and Q73 (feeling uncomfortable about eating in public -ve $P<0.0000005$). These are predominately psychological responses. No TMD symptoms were associated with stress at onset in this analysis.

2.4.2.j. Stress and TMD Symptoms.

Stressful events at onset were not associated with an increase in TMD symptoms. However, prolonged stress over the previous 5-8 years was higher in CFS patients with TMD symptoms, compared with CFS patients without TMD symptoms (CFS TMD= 64.0%, CFS No TMD=35.0% - $P<0.03$). The frequency of stressful events in the previous 5-8 years, was not different between the CFS patients with TMD symptoms and the CFS patients without TMD symptoms (CFS TMD= 80.0%, CFS No TMD=64.1% - P=NS). What was interesting was that 64% of the CFS patients with TMD symptoms asked for more information to be made available on coping with their illness and on stress management, compared with 35% of the CFS patients without TMD symptoms ($P<0.02$).

Prolonged stress was not associated with any difference in symptoms in CFS patients as assessed by standard regression analysis (Model: $R^2=0.374$, F=1.042, $P<0.44$), but was so with forward stepwise regression analysis (Model: $R^2=0.280$, F=2.910, $P<0.004$). The primary discriminating variable was muscle pain (-ve $P<0.03$), followed by poor appetite (-ve $P<0.04$) and fatigue with exercise (+ve $P<0.16$). Face pain was not an important variable. Forward stepwise regression analysis of the 12-month symptom frequency and 12-month symptom severity scores from the modified SCL-90-R, were both strong regression models ($P<0.00000$), but TMD symptoms were not associated with the presence of prolonged stress problems in CFS patients. Regression analysis of the clinically reported face pain and the 12-month frequency and 12-month severity of face pain and TMJ pain, were not associated with prolonged stress or the frequency of stressful events in CFS patients.

2.4.2.k. Antibiotics and TMD Symptoms.

CFS patients with facial pain had an increased prevalence of reporting antibiotic usage at onset compared with CFS patients without facial pain. The CFS group had an increased prevalence of patients who reported that they had received repeated prescriptions of antibiotics (CFS= 60.5%; C=35.6% - $P<0.002$) and having taken antibiotics for >4 weeks duration (CFS= 56.5%; C=23.0% - $P<0.00001$). These patients had increases in 1) prevalence of face pain (TMD= 59%; No TMD=35.3% - $P<0.04$); the 12-month frequency of face pain

(TMD=1.02±0.97; No TMD=0.62±0.95 – $P<0.04$); 3) the 12-month frequency of TMJ pain (TMD=1.39±1.06; No TMD=0.94±1.01 – $P<0.05$); and 4) the12-month severity of TMJ pain (TMD=1.37±1.23; No TMD=0.82±0.94 – $P<0.05$). Thus increases in antibiotic usage were associated with increases in TMD symptoms.

2.4.2.1. SCL-90-R Dimension Score Difference between CFS and Controls.

Mann Whitney U-test analysis of the SCL-90-R global index and dimension scores revealed that all measures were increased in CFS patients compared with controls (data not shown - all $P<0.0001$). Table 2.13 shows the regression analysis differences in the SCL-90-R dimension scores between CFS patients and control subjects. The main discriminate variables in the model were the somatization dimension, followed by the obsessive compulsion dimension. Depression and anxiety dimensions were not important inter-group determinants, although they were increased in the Mann Whitney U-test comparison. Thus the CFS and control subjects may be differentiated by the presence of physical and cognitive symptoms, rather than depression and anxiety symptoms.

2.4.3. SCL-90-R Dimension Score Associations with Facial and TM Joint pain.

2.4.3.a. Facial pain symptom frequency.

Table 2.13 shows the regression analysis of the dimension scores with facial pain frequency in the entire study population. Regression analysis found there was a large increase in SCL-90-R scores with the primary SCL-90-R dimension score being somatization followed by increased anxiety and reduced interpersonal sensitivity. Within the control group subjects who reported facial pain in the preceding 12 months were found to have an increased somatization dimension score ($P<0.008$) (Mann Whitney U-test).

Similarly within the CFS group the reporting of face pain in the preceding 12 months was associated with increases in the anxiety ($P<0.0002$), somatization ($P<0.002$), depression ($P<0.03$) and obsessive compulsion ($P<0.05$) dimension scores compared with the remaining CFS patients. Thus face pain frequency was predominantly associated with increased symptom reporting in all patients however within the CFS group increases in face pain were associated with increased anxiety and depression scores.

Table 2.13. Regression analysis of the differences in SCL-90-R dimension scores between the CFS and control groups and the study subjects with TMD symptoms compared with study subjects without TMD symptoms.

Analysis/Model	SCL-90-R Parameter	β(S.Err.β)	*P*
CFS v Control	Somatization	0.66(0.10)	$<1.0E^{-9}$
R^2=0.780	Obsessive Compulsion	0.47(0.10)	<0.00002
F=36.765	Paranoid Ideation	-0.11(0.07)	<0.13
P<0.00000	Hostility	0.27(0.07)	<0.0003
	PST	-0.53(0.14)	<0.0003
Individual TMD Symptoms			
Face Pain Frequency	Somatization	0.22(0.15)	<0.14
R^2=0.291	Anxiety	0.37(0.18)	<0.04
F=8.858	Interpersonal Sensitivity	-0.19(0.15)	<0.20
P<0.00000	Psychoticism	-0.17(0.14)	<0.22
Face Pain Severity	Somatization	0.37(0.16)	<0.02
R^2=0.354	Psychoticism	-0.24(0.12)	<0.04
F=9.578	Anxiety	0.50(0.18)	<0.007
P<0.00000	Obsessive Compulsion	-0.32(0.15)	<0.04
TMJ Frequency	Somatization	0.32(0.14)	<0.02
R^2=0.337	Anxiety	0.29(0.16)	<0.07
F=13.868	Paranoid Ideation	-0.25(0.10)	<0.01
P<0.00000	Hostility	0.14(0.11)	<0.22
TMJ Severity	Somatization	0.25(0.15)	<0.09
R^2=0.347	Paranoid Ideation	-0.36(0.10)	<0.0009
F=11.481	Depression	0.20(0.19)	<0.29
P<0.00000	Hostility	0.16(0.12)	<0.18
	Anxiety	0.19(0.17)	<0.26

2.4.3.b. Facial pain symptom severity.

Table 2.12 shows the regression analysis of the dimension scores associations with facial pain severity within the entire study population. Regression analysis found there was a large increase in SCL-90-R scores with the primary SCL-90-R dimension score being somatization followed by reduced psychoticism and increased anxiety. Increasing face pain severity correlated with the somatization (C – r=+0.35, *P*<0.006; CFS – r=+0.42, *P*<0.0001), anxiety (C – r=+0.28, *P*<0.04; CFS – r=+0.46, *P*<0.00002) and depression (C – r=+0.28, *P*<0.03; CFS – r=+0.27, *P*<0.02) dimension scores in both CFS patients and controls (Spearman rank). In addition face pain severity correlated with increasing obsessive compulsion and psychoticism dimension scores (both - r=+0.24 – *P*<0.03) in the CFS patients. Thus face pain severity was predominantly associated with increased symptom reporting as well as increased anxiety and depression scores in all patients.

2.4.3.c. TMJ pain symptom frequency.

Table 2.12 shows the regression analysis of the dimension scores with TMJ pain frequency in the entire study population. Regression analysis found there was a large increase in SCL-90-R scores with the primary SCL-90-R dimension score being somatization followed by increased anxiety and reduced paranoid ideation. Control subjects who reported TMJ pain in the preceding 12 months showed increased somatization dimension scores ($P<0.006$). CFS patients with TMJ pain showed no differences in any SCL-90-R index or dimensions score, compared with the CFS patients who did not report TMJ pain in the preceding 12 months (Mann Whitney U-test). Thus, frequency of TMJ pain was predominantly associated with increased symptom reporting in all patients.

2.4.3.d. TMJ pain symptom severity.

Table 2.12 shows the regression analysis of the dimension scores with TMJ pain severity in the entire study population. Regression analysis revealed a large increase in SCL-90-R scores with the primary SCL-90-R dimension score being somatization followed by reduced paranoid ideation and increased depression. Increasing TMJ pain severity in both CFS patients and control subjects correlated with the somatization (C – $r=+0.34$, $P<0.008$; CFS – $r=+0.35$, $P<0.001$), and depression (C – $r=+0.26$, $P<0.05$; CFS – $r=+0.23$, $P<0.04$) dimension scores. In addition TMJ pain severity correlated with increasing anxiety ($r=+0.31$ – $P<0.005$) and obsessive compulsion ($r=+0.29$ – $P<0.007$) dimension scores in the CFS patients. Thus TMJ pain severity was predominantly associated with increased symptom reporting as well as depression scores in all patients.

2.4.4. Post-exercise fatigue and TMD.

Muscle hyperactivity has been suggested to be associated with the aetiology of pain and TMD. Multiple regression analysis of the prevalence of post-exercise fatigue by the CFS patients was used to assess the association with their other reported symptoms. The standard regression analysis was strong (Model : $R^2=0.863$, $F=34.129$, $P<0.00000$) with positive associations found with muscle weakness ($P<0.0000000001$), difficulty with concentration ($P<0.0005$), sensitivity to foods ($P<0.02$), lymphodynia ($P<0.04$), patient perceived depression ($P<0.04$), and negative associations with sensitivity to smell ($P<0.01$), and earaches ($P<0.02$). No association was found between post-exercise fatigue and TMD or chronic pain. Thus post-exercise fatigue is associated with muscle weakness/fatigue and not muscle pain.

2.5. Discussion.

The chronic RDC/TMD type 1a pain patients in this study were compared with a control group which did not respond to a VAS of average pain intensity in the previous two weeks and did not report a history of or treatment for chronic pain in the previous 12 months. The RDC/TMD type 1a pain patients were predominately female (3:1 ratio) with a mean age between 30 and 40 years as has been reported in the literature for RDC/TMD type 1a pain patients (Schwartz, 1955; Campbell, 1958; Pedersen & Hansen, 1987). The chronic fatigue syndrome patients with TMD complied with these same age and sex parameters. Thus, the two study cohorts were consistent with previous studies reported in the literature and provide appropriate study cohorts from which conclusions may be drawn (Schwartz, 1955; Campbell, 1958; Fricton et al, 1985; Marciani et al, 1985; Cooper et al, 1986; Grosfeld et al, 1986; Egermark Eriksson et al, 1987; Pedersen & Hansen, 1987; Szentpetery 1987; Riolo et al, 1987).

This study found that CFS patients do have evidence of TMD, which has a higher prevalence to that reported in control groups as previously reported (McGregor et al, 1996a). The CFS cohort showed the anticipated differences between symptom reporting and the presence of TMD signs (De Knater et al, 1993; de Laat & van Steenberghe, 1985; Heloe & Heloe, 1977; Nilner, 1981; Solberg et al, 1977). Whilst 29% of the CFS patients and no control subjects reported TMD pain, TMD signs were reported by 69% of CFS patients. In the CFS study control population 19% of subjects reported TMD signs whilst none of the RDC/TMD type 1a study controls reported TMD signs. Those CFS patients who reported TMD symptoms, showed a higher frequency of positive responses and higher severity scores during the previous 12 months compared with the CFS patients who reported TMD signs in the questionnaire. This is also consistent with previous studies on TMD symptoms, where the number of palpable sites and the severity of the pain, determined which subject reported symptoms or pursued treatment (Kleinknecht et al, 1986) and where increased TMD symptoms occur in patients with fibromyalgia (Plesh et al, 1996). Assessment of musculoskeletal symptom reporting by the CFS control group, revealed that only 12% were asymptomatic, whilst 20% of the control subjects selected in the RDC/TMD type 1a pain patient cohort, were also asymptomatic. The increase in TMD symptoms in the CFS study control cohort, is likely to be a reflection of the strict exclusionary criteria used in the

RDC/TMD type 1a control selection process, compared with the fatigue selection criteria applied to selection of the CFS control cohort. Common musculoskeletal and fatigue symptoms occurred in over 50% of the control subjects in both study control groups, which is consistent with the high level of palpable muscle tenderness in the population (Seligman et al, 1988; De Kanter et al, 1993). Thus, whilst musculoskeletal and fatigue symptoms are common in control subjects in this study, the severity and frequency of occurrence of the symptoms are the prime factors that determine whether patients report these to clinicians.

The presence of TMD/RDC type 1a pain was associated with a wide body distribution of pain, which was prominent in the axial skeleton, but also extended to the limbs and anterior chest wall in patients with severe symptoms. The distribution of patients in this study group with fibromyalgia-like pain, myofascial pain and localised pain presentation, appeared skewed toward the fibromyalgia-like patients. Only 7% of TMD/RDC type 1a pain patients had pain localised to the face. Twenty-eight percentage of TMD/RDC type 1a pain patients had pain in the facio-scapulo-humeral area, which consistent with myofascial pain syndrome, whilst 65% of TMD/RDC type 1a pain patients had widespread pain distribution similar to fibromyalgia. This may reflect the method of patient selection, the difference in approach in obtaining data from patient reporting of whole body symptoms, or simply a random occurrence. However, every effort was made to request that patients report symptoms distant from the face that they may not normally report in a dental setting. Thus TMD/RDC type 1a pain patients have pain in most areas of the body, which is consistent with other reports (Dixon, 1948; Eggen, 1954; Forsberg, 1956; Carraro et al, 1969; Heloe, 1976; Weinberg & Lager, 1980; Eriksson et al, 1988; Turp et al, 1998). The CFS study cohort was selected because of a severe polysymptomatic illness with widespread pain distribution (Sharpe et al, 1991).

A sudden onset of the condition was reported by 30% of the RDC/TMD type 1a patients and 64% of CFS patients. First pain at onset of TMD symptoms in the RDC/TMD type 1a group occurred in the head in 47%, with 70% reporting a facio-scapulo-humeral distribution. However, 19% also reported pain to be first noticed in the low back. Most patients reported localised or regional pain at onset that progressed too more generalised pain that involved all four quadrants in 65% patients in this group. The CFS patients reported a sudden onset and a high proportion reported face pain initially and face pain was reported to occur when their condition was worsening. This relationship between face pain and symptom severity is consistent with the finding that CFS patients who have TMD symptoms have a higher frequency of reporting of symptoms.

In the RDC/TMD type 1a patients a stressful event at onset was reported by 21%. In the CFS cohort 29% reported stressful events at onset. No difference in prevalence of stressful events at onset was found between CFS patients with TMD signs/symptoms or CFS patients without TMD signs/symptoms using t-test or multiple regression analysis. The percentage of patients reporting stressful events at onset was similar to that in other studies (Fricton et al, 1985; Schiffman et al, 1992). CFS patients with TMD symptoms did report an increased prevalence of prolonged stress, but did not report an increase in the frequency of stressful events compared with CFS patients without TMD symptoms. However multiple regression analysis did not find any relationship between prolonged stress and TMD symptoms. The data from this study do not provide strong evidence linking stressful events at onset with TMD symptoms and are consistent with the weak relationship found by Schiffman et al (1992).

The association between stressful events and TMD has arisen due to the early hypotheses, which proposed stress to be a major factor in for TMD expression (Schwartz, 1955; Laskin, 1969, Yemm 1969a, 1969b; 1971a; 1971b). The study of CFS patients in this thesis, has allowed separation of these events as the patients were not selected based on their TMD presentation. Stressful events at onset appear to occur in all chronic polysymptomatic patients with a prevalence between 20 to 30% and are predominately associated with psychological responses and not strongly associated with TMD symptoms.

The CFS cohort was selected because of a sudden or definitive infectious onset, usually a virus-like condition. It was therefore interesting to note that infectious events were common at onset in the RDC/TMD type 1a group. Seventy-one percent of the RDC/TMD type 1a patients associated onset of their symptoms with one or more causally-related events, including upper respiratory influenza-like infection, low-grade diarrhoea, genitourinary infection, glandular fever or trauma (Table 2.4). RDC/TMD type 1a patients with a sudden pain/symptom onset, were more likely to report either an upper respiratory / influenza like infection or a genitourinary tract infection, compared with gradual onset RDC/TMD type 1a patients. RDC/TMD type 1a patients had increased reporting of infectious events during their lives; including chronic recurrent upper respiratory tract infections, genitourinary infections and appendectomies. The CFS patients with face pain were more likely to report use of antibiotics or an adverse drug-reaction at onset compared with CFS patients with no TMD. Antibiotic use was also reported by the sudden onset RDC/TMD type 1a group. Similarly, multiple antibiotic prescriptions in the CFS patients was associated with increased frequency of reporting of face pain. It is also of interest to note that TMD symptoms in the control subjects were associated with diarrhoea, similar to that found in the RDC/TMD type 1a patients and the 12-month frequency and severity of feverishness and night sweats in the control subjects (Table 2.10a). Similarly tables 2.9 and 2.10a show that in CFS patients an increased frequency and severity of feverishness and night sweats (Q108) and sore glands (Q103) were positively correlated with 12-month face pain and TMJ pain frequency and severity. These data support an association between TMD symptoms and infectious events in the control subjects, the CFS patients and the RDC/TMD type 1a group.

These data support the pilot study data, where infectious events were associated with TMD (McGregor et al, 1992; 1993). Myalgia and lethargy are common symptoms following interferon injection (Mahrle & Schulze, 1990; Reddy et al, 1997). Interferon-induced activation of the 2'5'A-synthetase/RNAase L enzyme system is associated with a reduction in protein synthesis and an increase in proteolysis that is consistent with the observed reductions in muscle protein and RNA levels in chronic pain patients (Young, 1970; Pacy et al, 1988; Bowles et al, 1993; Suhadolnik et al, 1994). A reduction in total muscle protein and RNA has been observed in CFS patients who have persistent enterovirus infection (Bowles et al, 1993; Gow et al, 1994). As muscle pain and fatigue are common host responses to both infections and trauma, and may be associated with protein turnover dysregulation (Mortimore & Poso, 1987), variations in the hosts genetics and the sites and organ systems affected by different infectious agents and cytokines, and may lead to variation in symptom expression.

The TMD/RDC type 1a pain patients also had increased multi-organ system symptoms, however in the CFS patients multiple regression analysis revealed that TMD symptoms were not associated with multi-organ system symptoms. In the CFS patients the presence of TMD symptoms correlated with many similar symptoms to that found in the RDC/TMD type 1a patients. Comparison of the CFS TMD symptoms and the control group TMD symptoms, revealed overlapping yet distinct symptom sets. This disparity in symptom correlation may be the result of the increased prevalence of the symptoms in the CFS patients compared with the control subjects or alteration in the infectious agent or the host's response. TMJ pain occurred more frequently in patients with higher face pain scores and was associated with more symptoms than facial muscle pain alone. Thus, increasing severity of face pain is associated with increased prevalence and severity of TMJ pain. These data suggest that whilst there is a relationship between increasing body pain, multi-organ symptoms and TMD symptoms, that the latter may be associated with a distinct common host response or aetiological factor due to the similarity between TMD symptom expression in both CFS patients and control subjects. This is very important as it supports the observations of Plesh et al (1996) who suggest that TMD is a distinct clinical entity that has increased prevalence in patients with fibromyalgia and CFS.

Increased spontaneous facial muscle EMG activity first noted by Jarabak (1956) and subsequently by Munro (1975) in TMD patients and by Travell & Simons (1993) in other chronic pain patients has lead to the development of the hyperactivity model for muscle pain. Sharav & Benoliel (1993) discussed the proposal that muscle hyperactivity may cause muscle pain especially the day following increased activity. Increased post-exercise fatigue is a common feature of CFS and in this study was associated with muscle weakness and not muscle pain or TMD symptoms. This is consistent with the conclusions of Lund et al (1991) that muscle hyperactivity is not associated with chronic pain.

The chronic RDC/TMD type 1a pain patients have increased prevalence of: 1) musculoskeletal, gastrointestinal, genitourinary and neurological symptoms consistent with a systemic condition; 2) symptoms and histories suggestive of nasopharyngeal, gastrointestinal and genitourinary infectious events, particularly in patients reporting a sudden onset; 3) low grade fever and lymphodynia; 4) reporting of pain in their sexual partners; and 5) infectious events occurring at the time of onset. The CFS patients have increases in fatigue, musculoskeletal, gastrointestinal and neurological symptoms (Sharpe et al, 1991) and an increase in TMD symptoms (McGregor et al, 1996a). Jaw muscle pain in the CFS study control subjects, was associated with other TMD symptoms, as well as gastrointestinal symptoms such as stomach cramps, diarrhoea and abdominal pain. TMJ pain in the CFS study cohort control subjects was not only associated with the gastrointestinal symptoms found for face pain, but infectious symptoms, fatigue, weakness, sleep disturbance, and psychological responses such as nightmares and panic attacks. In the CFS patients, facial muscle pain was associated with an increased prevalence of symptoms, predominantly associated with infectious symptoms and cognitive disturbances, but not gastrointestinal symptoms. The presence of TMJ pain in the CFS subjects was also associated with an increased number of symptoms including infectious symptoms, fatigue, weakness, cognitive disturbances, genitourinary infections, sleep disturbance and psychological responses (nightmares, phobic responses). Thus TMD symptoms are associated with infectious events in the CFS study group and are consistent with that seen in the RDC/TMD type 1a study group. This association is further supported by the association between increased pain symptoms in the sexual partners of the RDC/TMD type 1a patients and the observation of Heiberg et al (1980) that TMD symptoms do not occur at different rates between monozygous or dizygous twins.

A psychophysiological model has been proposed for chronic pain and several studies have shown in muscle pain patients an association with increased psychological dimension changes, particularly depression scores (Pelz & Merskey, 1982; Clark et al, 1985; Buckelew et al, 1986; Von Korff et al, 1988; Lee et al, 1989). In this study, both the RDC/TMD type 1a patients and the CFS patients had increases in SCL-90-R somatization, obsessive compulsion and depression dimensions. Multiple regression analysis found that both groups had increases in somatization and obsessive compulsion dimensions, but depression and anxiety were not important inter-group determinants. The failure to find depression as a major inter-group determinant was reported in CFS patients previously (McGregor et al, 1996c). In the RDC/TMD type 1a study cohort, depression and anxiety scores were not associated with those factors found by logistic regression to be important inter-group determinants, e.g. the VAS, symptom prevalence index or SCL-90-R questions 14 (fatigue / lethargy), 40 (nausea) and 42 (muscle soreness). Therefore, these data do not provide strong evidence for a direct association between increasing affective disturbance and muscle pain or physical symptom prevalence in either the RDC\TMD type 1a or the CFS study cohorts. Table 2.6 shows that SCL-90-R responses can be interpreted as either an affective psychological response or a physical symptom of a disease with some possible psychological consequences. To assess these potential interpretations, other methods of analysis need to be used, that are not part of this study. The SCL-90-R responses do suggest that chronic pain and CFS patients have an increased prevalence of altered cognitive responses, particularly associated with memory and concentration. These data are interesting since alterations of N-methyl-D-Aspartate (NMDA) receptors in brain and spinal cord are not only associated with modulation of pain responses (Corderre, 1993; Nasstrom et al, 1992), but also memory functions (Venable et al, 1990). The possibility of changes in brain metabolism and function are also supported by the observation of alteration of the hypothalamic-pituitary-adrenal axis in fibromyalgia syndrome (McCain & Tilbe, 1989), depressive and chronic fatigue syndrome patients (Cleare et al, 1995). Whilst depression is suggested to be associated with serotonin deficiency, TMD symptoms and CFS appear to be associated with alterations in dopamine/noradrenaline (Lynch et al, 1961; Malkin, 1964; Evans, 1965; Shapiro et al, 1965; Hiatt & Schwartz, 1966; Kraak, 1967; Ryan et al, 1968; Bell, 1969; Pertoutka et al, 1988; Demitrack et al, 1989).

2.6. Conclusions.

The results of this study indicate that in CFS patients with TMD symptoms and chronic RDC/TMD type 1a pain patients: 1) muscle pain symptoms are not restricted to the orofacial areas; 2) multi-organ involvement and diverse symptom expression suggest that a systemic condition may be occurring in at least a subgroup of patients; 3) gastro-genitourinary symptoms are prominent; 4) onset appears to be associated with infectious events; 5) there is an increased prevalence of history of infectious events and recurrent infections; and 6) partners of muscle pain patients appear to have an increased prevalence of muscle pain. An assessment of the biochemical changes is warranted and an assessment of alterations in amino acid metabolism is the basis of chapter 3.

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Chapter 3.

3. TMD and Changes in Biochemistry, Amino and Organic Acid Metabolism.

3.1. Introduction.

Chapter 1 details the pilot study data of 35 chronic RDC/TMD type 1a pain patients, which found an association with multi-organ symptoms and a history of urinary tract infection (McGregor et al, 1992). These patients had an increased prevalence of elevated midstream urinary staphylococcal counts (P<0.02) compared with age and sex matched controls (McGregor et al, 1993) and preliminary tests showed an association between the toxicity of these organisms and pain expression. Chapter 2 confirmed the pilot study association between RDC/TMD type 1a pain and multiple body symptoms and infectious events, and also established an association between TMD and alterations in cognitive functions. However multiple regression analysis revealed that TMD symptoms were clustered with a distinct group symptoms that had an increased prevalence in patients with multiple body symptoms but were unrelated to the majority of symptoms that also increased in the polysymptomatic patients. This suggests that the cluster of symptoms associated with TMD symptoms have a distinct molecular basis, which is consistent with the hypothesis proposed by Plesh et al (1996).

Russell et al (1989) found alterations in serum amino acids and proposed a serotonin-based hypothesis for chronic pain (Russell et al, 1992). Conversely, alterations in jaw muscle tone, one feature of TMD, has been associated with alterations in dopaminergic activity (Lynch et al, 1961; Malkin, 1964; Evans, 1965; Shapiro et al, 1965; Hiatt & Schwartz, 1966; Kraak, 1967; Bell, 1969; Sunden Kuronen et al, 1983a, 1983b; Pertoutka et al, 1988). Infections and infection-associated cytokine activity are associated with changes in amino acid metabolism (Mortimore & Poso, 1987; Hasselgren, 1995; Jeevanandam, 1995) and painful muscle has been found to have reduced total protein and RNA levels (Young, 1970; Pacy 1988; Bowles et al, 1993). In CFS patients, symptom expression has been associated with increased RNase-L activity, the activation of which is usually an cytokine-associated event (Suhaldonik et al, 1994; 1997). This increased RNase-L activity is associated with reduced RNA levels and subsequently reduced protein synthesis and a reduction in muscle protein and RNA as already noted in painful muscle (Young, 1970; Pacy 1988; Bowles et al, 1993). These data provide a body of evidence that suggests that evaluation of amino acid metabolism associated with infectious symptoms in TMD patients be warranted.

This chapter assesses standard blood cell and biochemistry, amino acid metabolism and alterations in RNase-L, soluble interleukin-2 receptor (sIL-2r) and interleukin-6 (IL-6) in relationship to TMD patients. The data was obtained from; a) a study group with defined RDC/TMD type 1(a) (Le Resche et al, 1992); b) a second study group with defined chronic fatigue syndrome (Sharpe et al, 1991) as in Chapter 2; and c) a third CFS study group, independently obtained, to assess the association between TMD and RNase-L, sIL-2r and IL-6. The aim of evaluation of the CFS patient groups and the defined RDC/TMD type 1a group is to determine the relationship between TMD symptom expression and the biochemistry and to reduce the potentially confounding factors that may relate to symptom expression in other parts of the body as demonstrated for symptom expression in chapter 2.

3.2. Literature Review.

3.2.1. Overview of Amino Acid Metabolism.

Figure 3.1 shows an overview of human amino acid metabolism. Dietary protein, microbial produced amino and biogenic amine compounds as well as protein components of human excretory products, such stomach and pancreatic enzymes, are absorbed from the gut. The amine compounds enter the portal blood circulation where they are delivered to the liver (pathway 1 figure 3.1). The liver detoxifies the biogenic amines and processes the amino acids. Some amino acids are stored in the liver whilst others are released into the circulation where they are transported into the peripheral tissues for usage in protein synthesis and energy provision (pathway 3, Figure 3.1). Utilisation of the amino acids for energy provision occurs by degradation into the glycolytic and citric acid pathways, with the excess resultant nitrogen entering the urea cycle for conversion to urea (pathway 2, Figure 3.1). Excess nitrogen (as urea) is excreted from the kidney (pathway 4, Figure 3.1). During normal metabolism, protein degradation is balanced by protein synthesis and excess nitrogen from the tissues is returned to the liver as either alanine or glutamine (Lehninger et al, 1993). However during periods of increased energy requirement, such as infection, increased exercise, stress or other illness, muscle, adipose tissue and connective tissues undergo increased proteolysis to provide amino acids for the period of increased energy demand (Mortimore & Poso, 1987).

Disposal of the excess ammonia and nitrogen to the liver from catabolism of amino acids within muscle occurs via release of alanine and glutamine from muscle (Figure 3.2). This reaction is mediated by the enzyme alanine aminotransferase (ALT) in both muscle and liver and is intimately involved with glycolysis and gluconeogenesis in both muscle and liver (Lehninger et al, 1993).

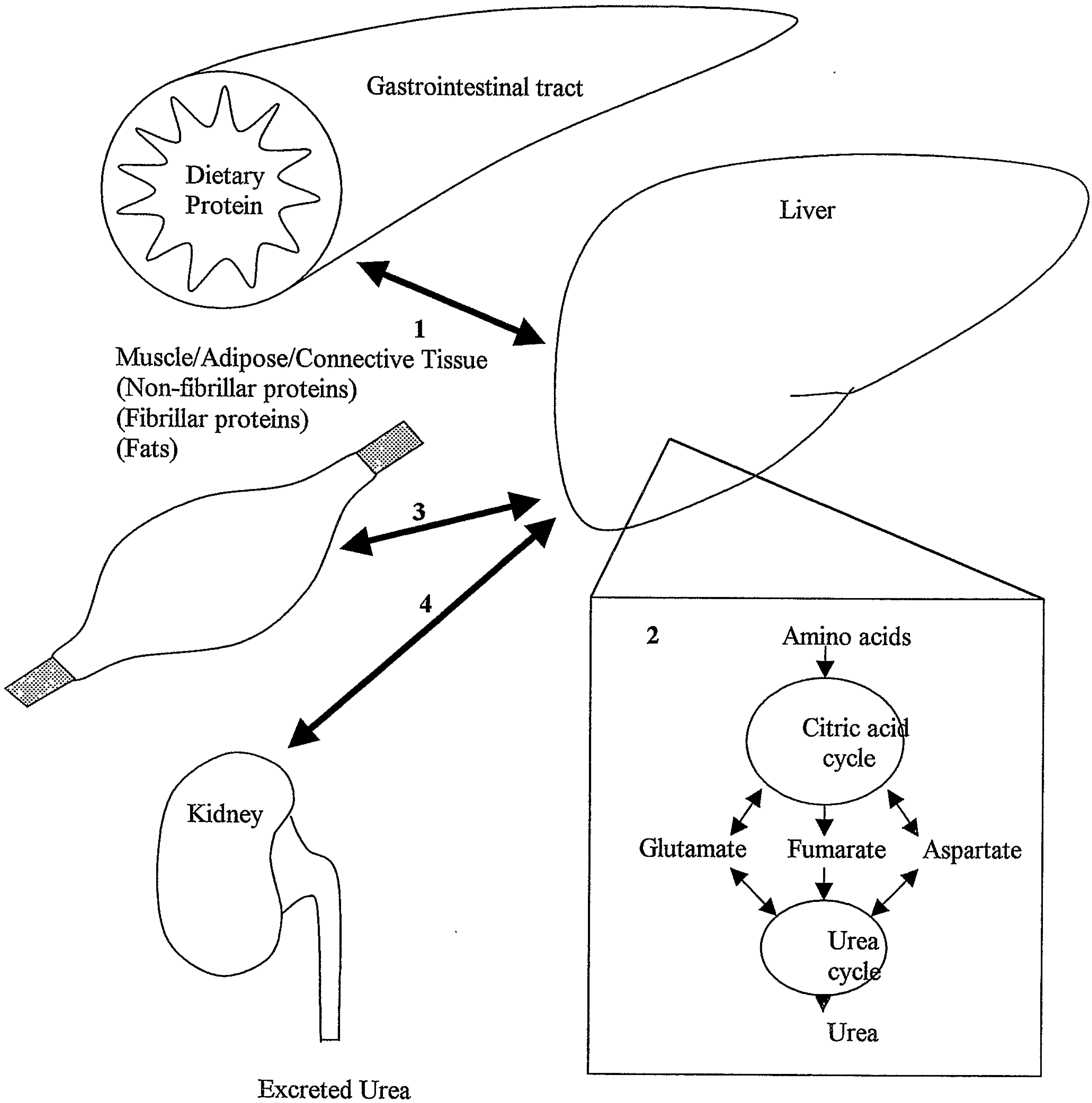


Figure 3.1. Overview of Human Amino Acid Metabolism.

1. Protein and amino acids of dietary, host secretory and microbial origin are absorbed from the gastrointestinal tract.
2. The basic biochemical mechanisms that result in urea production and ammonia removal.
3. Protein synthesis and proteolysis regulate the serum amino acid levels with the muscle, adipose and connective tissue acting as the reservoirs of amino acids.
4. Urea is transported to the kidney for removal – reducing excess nitrogen.

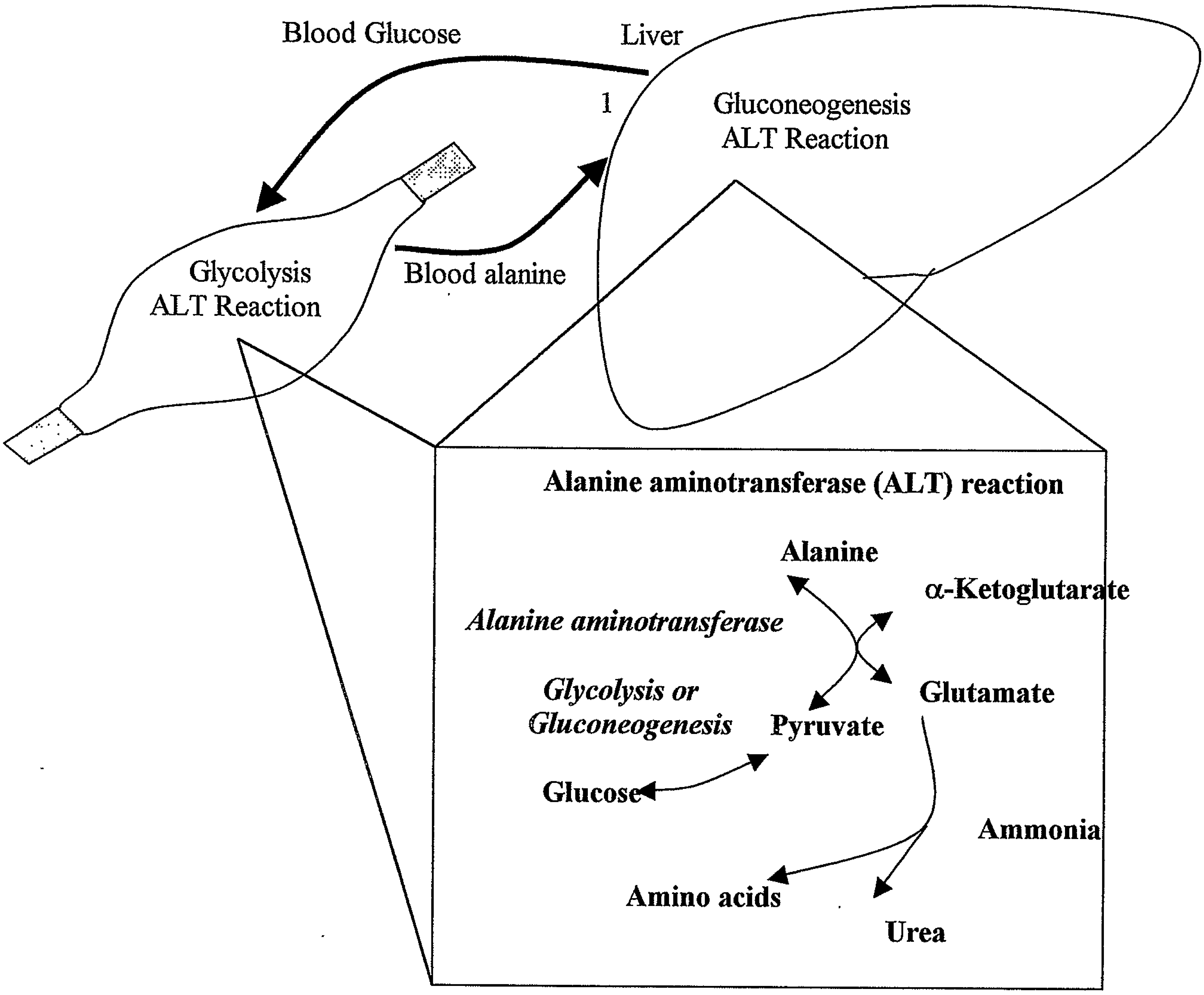


Figure 3.2. Overview of Glucose/Alanine and Alanine Aminotransferase (ALT) Metabolism.

1. In the liver alanine is used in the gluconeogenesis reaction to produce glucose which is than either stored as glycogen or enters the blood stream to maintain blood glucose levels.
2. As muscle protein is degraded, amino acids are released, which may be metabolised and will release ammonia that is converted to glutamate and free alanine by the ALT reaction. Alanine and glutamine are released into the circulation to return to the liver to release ammonia by the ALT reaction with the ammonia being used to form amino acids or urea.

The net result is the removal of ammonia and excess nitrogen from muscle. Alterations in the ALT reaction and its precursors and/or products may indicate alterations in amino acid oxidation in either liver or muscle.

Figure 3.3 shows the detail of the actions that take place in the liver cell mitochondria and cytoplasm for the disposal of the excess nitrogen and ammonia. The relationship between the mitochondrial activity and urea cycle function is shown and involves both ALT and aspartate aminotransferase (AST) activity.

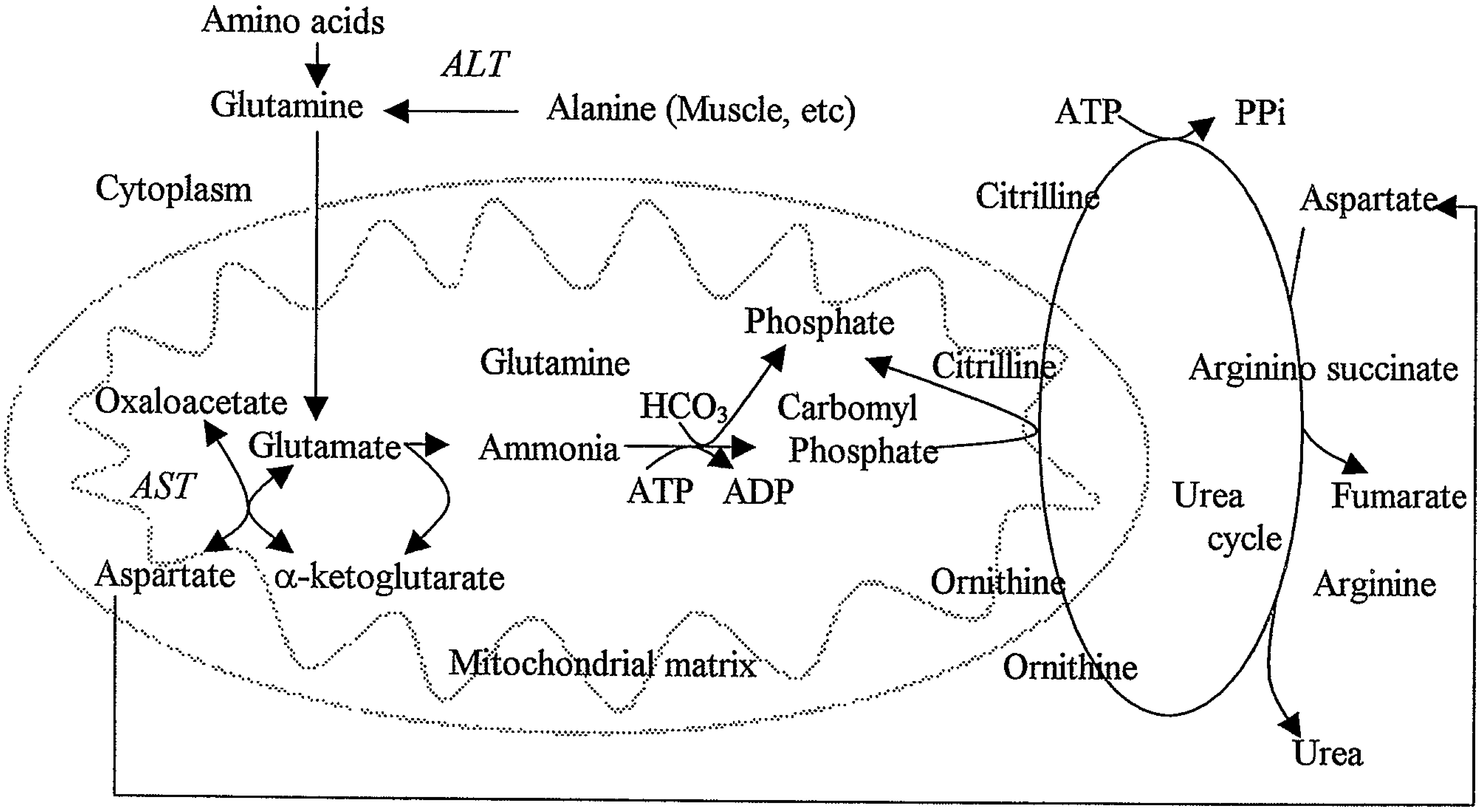


Figure 3.3. Overview of mitochondrial and cytoplasmic urea cycle reactions for ammonia removal.

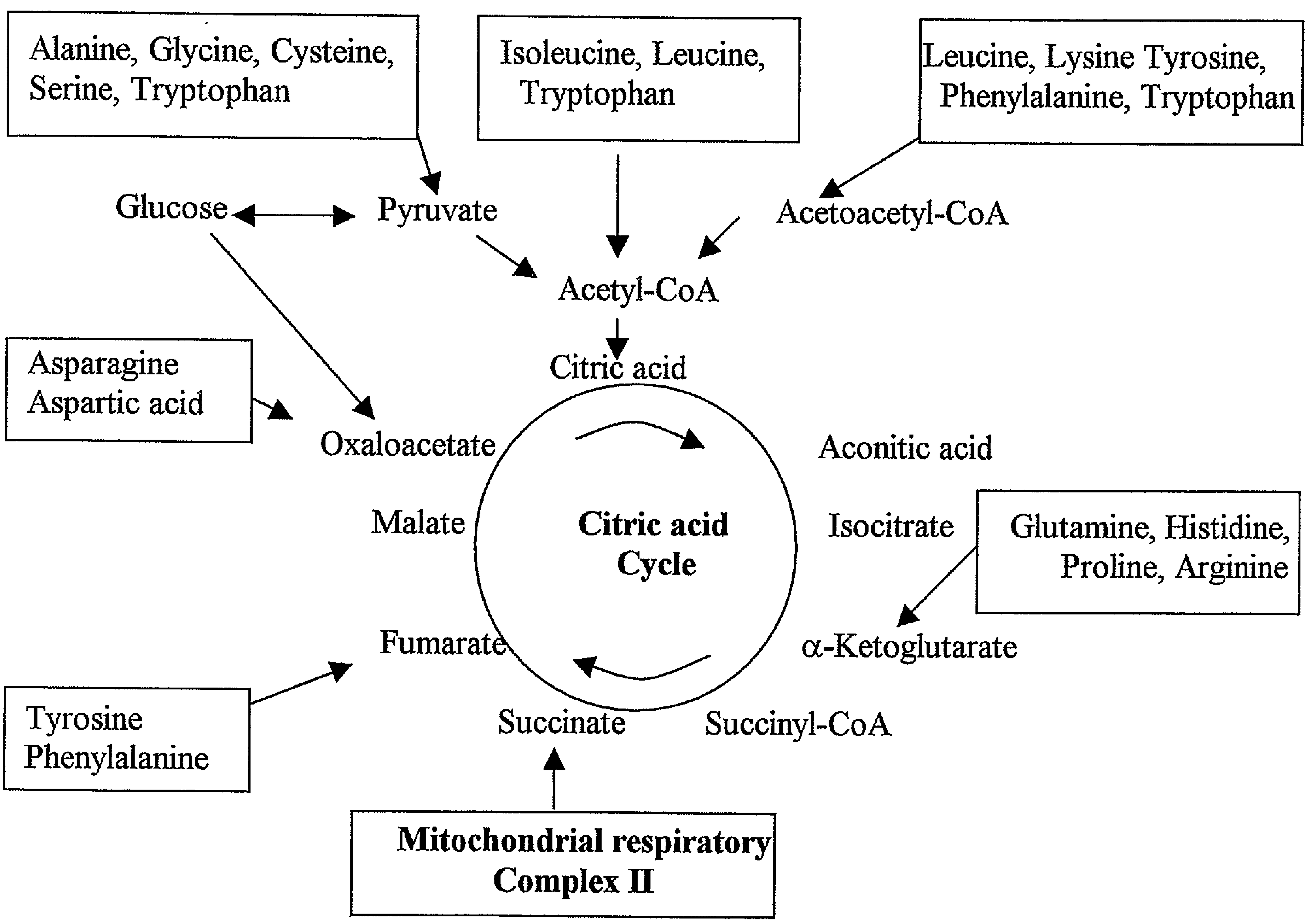


Figure 3.4. Summary of the metabolism of the oxidative positions of the various amino acids for provision of energy within the citric acid cycle and glycolysis.

Figure 3.4 shows the positions in the citric acid cycle and glycolytic pathways at which catabolism of the various amino acids provide intermediates for energy production. Garber et al, (1976) showed that both alanine and glutamine release from muscle occurred following administration of cysteine, leucine, valine, methionine and isoleucine, whilst increased alanine release alone occurred with administration of aspartate, serine, threonine and glycine. Increases in glutamine alone occurred with administration of tyrosine, phenylalanine and lysine. Therefore differences in the availability of aspartate, serine, threonine, glycine, tyrosine, phenylalanine and lysine will be reflected in differences in blood alanine and glutamine levels. Those amino acids which provide increases in alanine levels feed intermediates into the production of pyruvate and oxaloacetate, whilst glutamine levels are influenced by those amino acids that provide intermediates as acetoacetate or fumarate.

At rest, 6 amino acids are metabolised in muscle (leucine, isoleucine, valine, asparagine, aspartate and glutamate), and provide the ammonia to synthesise alanine and glutamine (Wagenmakers, 1998). The ALT reaction functions to maintain the citric acid cycle intermediates, particularly during the first 10 minutes of exercise and once glycogen is depleted, leucine is oxidised to acetyl-CoA to provide energy intermediates. Exercise-associated muscle fatigue occurs when both glucose and leucine are reduced (Wagenmakers, 1998). Following initiation of exercise, citric acid cycle intermediates are increased, particularly succinate, malate and fumarate, which constitute >90% of the increased citric acid cycle intermediates (Gibala et al, 1997). This increase in intermediate products is associated with increases in alanine and pyruvate and a reduction in glutamine, indicating a primarily glycolysis/ALT-based energy provision and not the ammonia/amino acid-based glutamate dehydrogenase reaction. Thus the ALT reaction along with alterations in citric acid cycle components in the last third of the citric acid cycle (succinate to oxaloacetate) and the amino acids (asparagine, aspartate, tyrosine, phenylalanine) that are oxidised into that part of the cycle, are likely to be good indicators of glycolytic-based fatigue mechanisms. The components of the last third of the citric acid cycle are also those which constitute part of the argininosuccinate-aspartate shunt of the citric acid cycle that links the citric acid and urea cycles.

Figure 3.5 shows the argininosuccinate-aspartate shunt of the citric acid cycle and its association with mitochondrial components, the citric acid and urea cycles, and nitric oxide production. Figure 3.5 shows the importance of the increase in fumarate and malate seen in early exercise activity (Gibala et al, 1997) for the appropriate activity in both the citric acid and urea cycles. The increase in the urea cycle is associated with an increased potential for the production of nitric oxide (Albina & Mateo, 1995). Nitric oxide is a second messenger molecule which is increased during inflammatory events and if produced in increased amounts for long periods may induce tissue damage (Albina & Mateo, 1995). Thus the ALT reaction along with the amino acids involved in the argininosuccinate-aspartate shunt and its associated citric and urea cycles (succinate, malate, oxaloacetate, asparagine, aspartate, tyrosine, phenylalanine), are likely to not only be good indicators of glycolytic-based fatigue mechanisms, but also those associated with nitric oxide production. If there is loss of precursors for nitric oxide production, the ability to mount an appropriate response may be faulty, which in turn may lead to alteration in host metabolism.

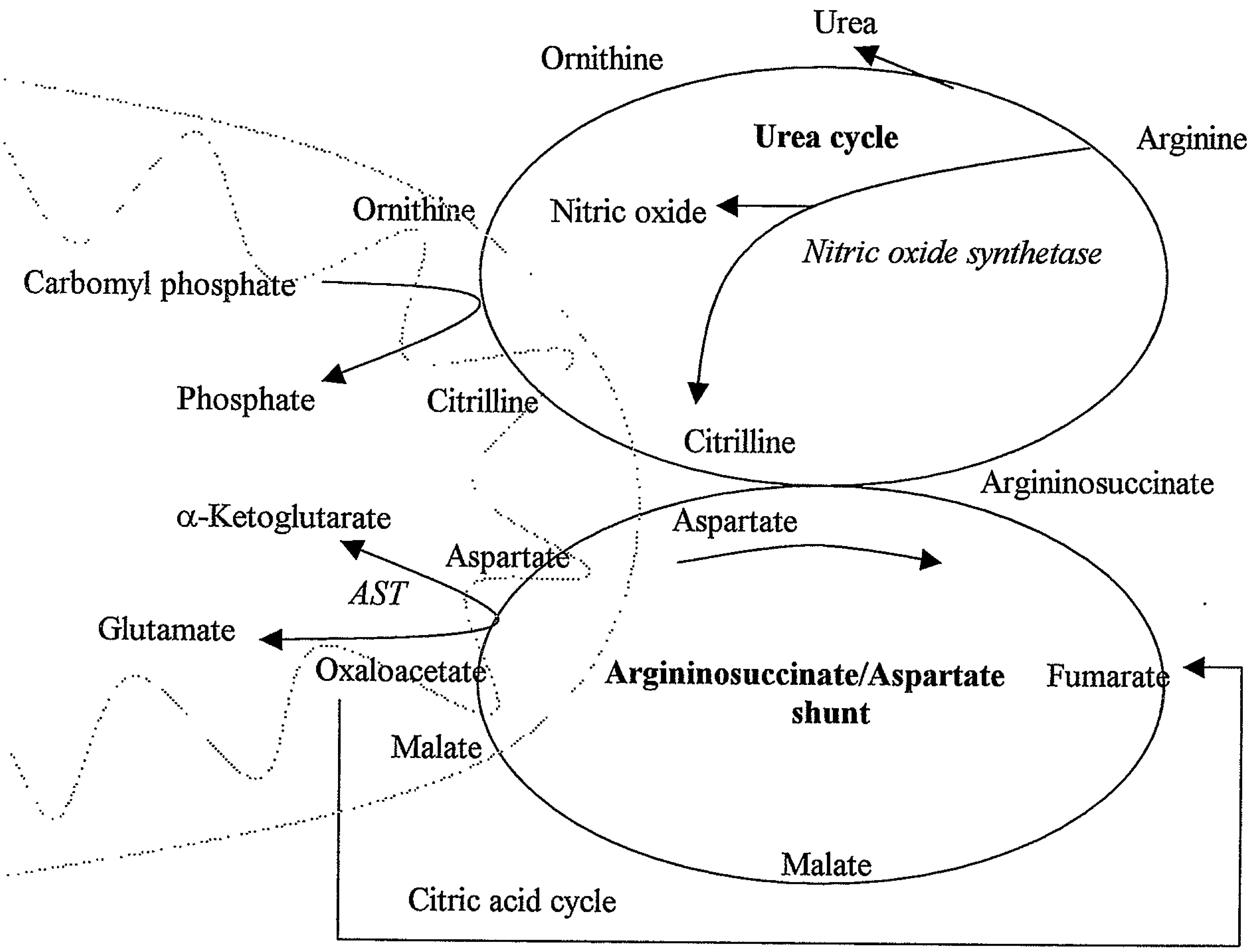


Figure 3.5. Nitric oxide production, the urea cycle and argininosuccinate-aspartate shunt of the citric acid cycle.
The provision of arginine for the production of nitric oxide and the removal of citrilline after nitric oxide production are important events controlled by the enzymes of the urea cycle. These events are also intricately involved with the activity of the citric acid cycle by the argininosuccinate-aspartate shunt. Fumarate, malate and oxaloacetate are all from the last third of the citric acid cycle. Fumarate, malate and succinate are the components that are increased during the initial stages of exercise. The amino acids that are catabolized to provide energy to this section of the citric acid cycle are aspartate, asparagine, phenylalanine and tyrosine.

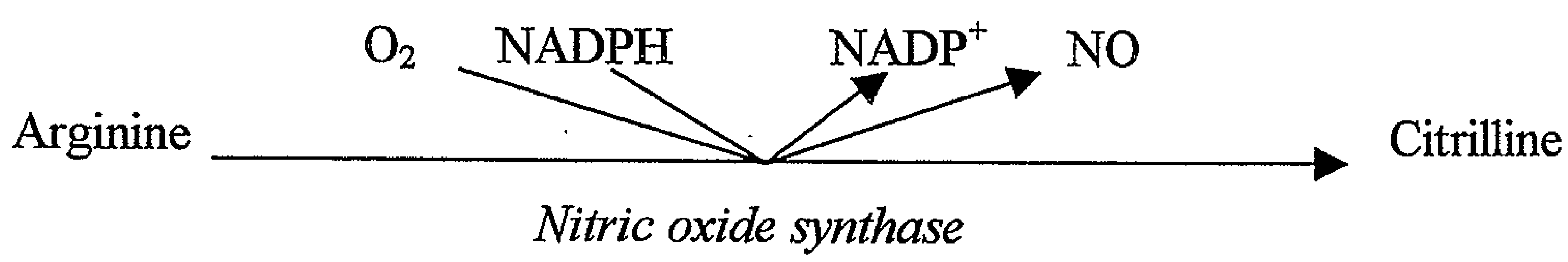
It has been proposed that increased tissue damage occurs with prolonged exercise as the latter is associated with increased plasma levels of ALT, AST, creatine kinase, leukocyte counts and cytokine levels (Kayashima et al, 1995; Mena et al, 1996; Bruunsgaard et al, 1997). Chronic strenuous exercise over a 13 week military training program resulted in increases in the white cell count which positively correlated with increases in AST, ALT, lactate dehydrogenase, urea, creatine kinase, superoxide dismutase and uric acid. This suggested that combined muscle and liver damage was occurring along with upregulation of the urea cycle (Kayashima et al, 1995). Bruunsgaard et al (1997) found an increase in interleukin-6 (IL-6), lymphocyte numbers (natural killer cells, CD8 suppressor t-cells) and creatine kinase levels after "eccentric" exercise (contraction with limitation of limb movement), which causes more profound muscle tissue damage, but not normal or "concentric" exercise (contraction with normal limb movement). Conversely, the number of neutrophils increased after concentric exercise but not eccentric exercise. Bruunsgard et al (1997) concluded that an alteration in plasma catecholamines appeared more important in leukocyte recruitment and that the increased tissue damage seen with eccentric exercise was associated with the alterations in the different types of leukocytes recruited between the two forms of exercise. Thus chronic exercise is associated with variable alterations in immune cell numbers and characteristics, which in turn are associated with increased tissue damage (ALT, AST and urea cycle components). TMD patients require investigation to assess whether immune cell and tissue damage occurs with chronic TMD and/or jaw muscle pain. Vega et al (1998) showed that acute injury to limb tissue, using tourniquet application, resulted in increases in serum ALT and AST, as well as nitrate, which is a marker of increased nitric oxide production. Inhibition of nitric oxide production resulted in further increases in serum ALT and AST, whilst enhancement of the nitric oxide response resulted in reduced ALT and AST. They concluded that endogenous nitric oxide production protected the liver from oxidative stress in acute tourniquet shock. As with prolonged exercise (Kayashima et al, 1995; Mena et al, 1996; Bruunsgaard et al, 1997), neutrophil levels increased following transient liver ischaemia-reperfusion (Liu et al, 1998). Inhibition of the nitric oxide response was associated with an increased neutrophil accumulation, superoxide production and liver damage (Liu et al, 1998), which was mediated by neutrophil associated mechanisms independent of nitric oxide production (Wagner et al, 1996). Thus a blunted or inhibited nitric oxide response associated with increased neutrophil superoxide concentrations was important in the increase in tissue damage in transient liver ischaemia-reperfusion (Liu et al, 1995). This is consistent with prolonged exercise-associated tissue changes (Kayashima et al, 1995; Mena et al, 1996; Bruunsgaard et al, 1997). Other immune mediation factors, such as histamine, may also increase neutrophil adhesion and tissue damage by activation of histamine H1 and H2 receptors but not H3 receptors (Schaefer et al, 1998).

In chapter 2 TMD symptoms were found to have a high association with infectious events and not to be associated with post-exercise fatigue. Similarly the pilot study (Chapter 1) suggested an associated between staphylococcal toxicity and TMD symptoms. Liaudet et al (1997) and Vos et al (1997) both reported that bacterial toxins induce toxic shock as well as tissue and liver damage associated with increases in ALT, AST and nitric oxide production. Toxic shock results from an increase in the production of nitric oxide from L-arginine, and toxin-associated tissue damage (increased serum ALT, AST, urea, creatinine, and lactic acidaemia) and reduced vascular tone (hypotension). Interestingly the amino acid, L-lysine, inhibits L-arginine uptake through the shared amino acid transporter (system y+) and inhibits lactic acidaemia and reduces the organ injury found with endotoxin (Liaudet et al, 1997). It appears that bacterial toxin and cytokine driven changes to urea cycle activity may induce the same changes as noted with prolonged exercise or ischaemia-reperfusion. This suggests that alteration in urea cycle and its associated argininosuccinate-aspartate shunt may play a role in regulation of nitric oxide production and toxicity reactions. Thus the pilot study observation of an association between staphylococcal toxicity and TMD (chapter 1), and the evidence of the association between infectious events and TMD (chapter 2) support an alteration in amino acid metabolism associated with the urea cycle, argininosuccinate-aspartate shunt and citric acid cycle.

Many of the symptoms of CFS patients have been attributed to increased immune cell activation resulting in dysregulated cytokine excretion (sIL-2r, IL-6, Tumour necrosis factor - TNF) (Buchwald et al, 1997). Thus the alterations in leukocytes noted by Bruunsgaard et al (1997), Kayashima et al (1995) and Mena et al (1996) may produce cytokines or other inflammatory mediating substances, which are associated with symptom expression in prolonged exercise. Bruunsgaard et al (1997) reported that increases in lymphocytes and IL-6 levels occurred with eccentric exercise whilst concentric exercise was associated with neutrophil levels suggesting that different inflammatory cytokine increases may not only be associated with muscle pain but may also occur as a result of the condition. Thus assessment of cytokine activity in chronic TMD patients is warranted.

3.2.2. An Overview of Nitric Oxide, Amino Acid Metabolism and Pain.

Nitric oxide is produced from a reaction converting arginine to citrilline (two components of the urea cycle) by the enzyme nitric oxide synthase. (Also known as NADPH Diaphorase; E.C.1.14.13.39).



Two types of nitric oxide synthase exist: 1) constitutive or continually present in tissues (Nitric oxide synthase 1 in neurones; Nitric oxide synthase 3 in endothelial cells); and 2) inducible (Nitric oxide synthase 2a, induced by endotoxins and cytokines). Both types require O_2, flavin adenine dinucleotide (FAD), flavin mononucleotide (FMN), nicotinamide adenine dinucleotide phosphate (NADPH) and tetrahydrobiopterin as cofactors. Activation of the constitutive types are calcium and calmodulin dependent (reviewed in Albina & Mateo, 1995), whilst the inducible form is found in leukocytes, astrocytes, hepatocytes, endothelial cells and fibroblasts.

Figure 3.5 shows the relationship between nitric oxide production by nitric oxide synthase and the urea cycle. Once produced nitric oxide is rapidly converted to the stable compounds NO^-_2 and NO^-_3 (Albina & Mateo, 1995). The type of reactive metabolite produced with activation of nitric oxide production is determined by the redox potential of the cell in which it is produced (Lipton et al, 1993) and has the paradoxical outcome of being either protective or toxic. The active by-products of nitric oxide formation react with atoms or molecules that contain unpaired electrons, such as oxygen, superoxide and metal ions (iron, copper, and manganese). Various active nitric oxide by-products combine with iron in haem proteins, such as haemoglobin, with the resultant production of the second messenger molecule, cyclic-Guanosine 5'-monophosphate (c-GMP). Nitric oxide by-products can inactivate enzymes with iron-sulphur prosthetic groups and tyrosine residues. Target enzymes and molecules include the citric acid cycle and mitochondrial electron transport associated enzymes, aconitase, ubiquinone and succinate oxidoreductases as well as ferritin, ribonucleotide reductase, AMP deaminase, and the glycolysis/gluconeogenesis enzyme glyceraldehdye 3-phosphate dehydrogenase (Albina & Mateo, 1995). Thus nitric oxide has profound effects upon many enzyme systems and molecules and is associated with many physiological changes including neural, cardiovascular, pulmonary, renal, gastrointestinal and endocrine functions (reviewed in Albina & Mateo, 1995), and may have protective and destructive actions.

Pain responses in both the central nervous system (Coderre, 1993; Coderre & Yashpal, 1994; Wong et al, 1998) and peripheral tissues (Holthusen & Arndt, 1995; Kang et al, 1995; Lorenzetti & Ferreira 1996) are associated with increased nitric oxide production. Coderre & Yashpal (1994) showed that activation of receptors or cellular mechanisms that increase the intracellular messengers, nitric oxide, arachidonic acid and protein kinase C lead to persistent nociception in response to tissue injury in rats. Central sensitisation induced by excitatory amino acids and substance P was reversed by pre-treatment with nitric oxide, arachidonic acid and/or protein kinase C inhibitors. These nitric oxide-related changes are linked with a decrease in the detection and tolerance thresholds of pain in humans (Thomsen et al, 1996) and may be initiated by a number nitric oxide radical generating mechanisms (Machelska et al, 1997; Aley et al, 1988). The primary factor associated with this alteration in the hyperalgesic pain threshold may be the N-Methyl-D-Aspartate (NMDA)-associated inhibition of $Na^{+}K^{+}$ATPase activity (Tavalin et al, 1997). These receptor associated events mediate a process called excitotoxicity (Thomas 1995) which in the nervous system is commonly associated with increased action of excitatory amino acids, glutamic and aspartic acids, on NMDA and similar receptors. Hyperalgesia mediated by NMDA and α-amino-3-hydroxy-5-methyl-4-isoxazole propionate (AMPA) receptors is linked with nitric oxide accumulated in a population of small cells and neuropil (an area of intermingled neuronal processes associated with synaptic junctions) in laminae II and III of the dorsal horn, consistent with a role in the processing of nociceptive signals (Morris et al 1994; Hama & Sagen, 1994). Interestingly, Siegan et al (1996) showed that implanting adrenal medullary cells into the spinal cord subarachnoid space resulted in reduction of hyperalgesia and allodynia, suggesting that alterations in opoids and catecholamines (dopamine, noradrenaline, adrenaline) may modulate the spinal nitric oxide associated pain mechanisms. Altered nitric oxide production in the central nervous system has been suggested to be involved in phantom limb pain (Neidbala et al, 1995) and neuropsychiatric disorders (Karatinos et al, 1995). Apart from peripheral nociceptive activation, bacterial lipopolysaccharide (LPS) (Wiertelak et al, 1994a, Watkins et al, 1994) and interferon-γ (IFN-γ) (Xu et al, 1994) associated with inflammatory events can also activate the spinal column NMDA-nitric oxide cascade and induce hyperalgesia. Watkins et al (1994) also showed that substance P, cholecystokinin and excitatory amino acids acting at sites other than NMDA, serotonin or kappa opiate receptors, mediate this bacterial LPS associated spinal hyperalgesia. Thus nitric oxide, excitatory amino acids and catecholamines have a complex role in the modulation of pain responses in the central nervous system.

Activation of peripheral nociception is also involved in pain mechanisms. Peripheral stimuli such as the herniation of a cervical intervertebral disc (Holthusen & Arndt, 1994) or the injection of nitric oxide into peripheral tissues are associated with increased pain (Kang et al, 1995). Kawabata et al (1994) assessed the peripheral activity of nitric oxide on formalin induced hyperalgesia. Increased arginine levels facilitated the second phase of nociceptive responses at low doses but had antinociceptive activity in higher doses. Conversely, inhibition of nitric oxide production reduced the nociceptive response of the second phase. Thus variation in nitric oxide activity modulates peripheral nociceptive activity with reduced levels or blunted responses leading to enhanced nociceptive activity. These activities are separate from the central nervous system activities (Wiertelak et al 1994b) and the same pain associated modulatory actions of nitric oxide concentration are not seen in the spinal cord (Malmberg & Yaksh, 1993). Also of significance in peripheral tissues are the roles nitric oxide scavengers such as haemoglobin and amino acids, which by binding nitric oxide radicals modulate nitric oxide activity or availability (Inoue et al, 1998). Thus in peripheral tissue the levels of nitric oxide and the availability of nitric oxide scavengers, such as haemoglobin, may modulate the pain response in a different manner to that seen in the central nervous system.

Lipton et al, (1993) found that nitric oxide activation in neural tissue can result in the paradoxical outcome of either neuroprotective or neurotoxic activity. This change in activity was associated with the redox-state of the neurone. Activation of NMDA receptors along with nitric oxide production and superoxide anion presence lead to the formation of peroxynitrate ($ONOO^-$) and neurotoxicity. Conversely, down-regulation of NMDA receptors along with nitric oxide production and the formation of NO^+ are neuroprotective. These events were regulated by the redox status of the cell and have also been shown to have similar protective and toxic activities in bacterial cells (De Groote et al, 1995). Interestingly, the prolonged activation of the nitric oxide response is associated with post-transcriptional modulation of nitric oxide synthase activity (Zhang et al, 1998), which was associated with an 80% decrease in the redox control protein, thioredoxin, and a 27% decrease in thioredoxin mRNA expression. Thioredoxin is a redox control protein that is produced in response to cellular redox stressors and is an important intracellular redox control system (reviewed in Nakamura et al, 1997). Thus nitric oxide is able to modulate the redox-state of cells by changes in redox protein levels, which in turn may result in differences in the cellular production of nitric oxide by-products and quite different molecular outcomes.

Nitric oxide production, the redox status and Na^+K^+ATPase function may alter neuronal excitotoxicity and pain responses. Sato et al (1997) found that the inhibition of Na^+K^+ATPase by nitric oxide was associated with the oxidation state of the cell with sulphur reducing agents (reduced glutathione, cysteine) or oxide scavenging agents (ascorbic acid) able to inhibit the effect of nitric oxide. Once Na^+K^+ATPase was inhibited by nitric oxide only the SH reducing agents were able to reverse the inhibition. The production of different nitric oxide radicals was associated with different patterns of Na^+K^+ATPase inhibition. Superoxide associated peroxynitrate induced Na^+K^+ATPase inhibition but was attenuated by nitrogen dioxide whilst nitrogen dioxide in the absence of superoxide induced Na^+K^+ATPase inhibition. Thus nitrogen dioxide had both agonist and antagonist effects on Na^+K^+ATPase activity which was dependent upon the presence of other Na^+K^+ATPase active metabolites. Boldyrev et al (1997) showed that nitric oxide radials combined with iron complexes were the most potent inhibitors of Na^+K^+ATPase. Restoration of the sulphur groups on the Na^+K^+ATPase using cysteine restored the activity. Thus the activity of Na^+K^+ATPase is significantly influenced by nitric oxide radicals and the redox potential of the cell and may play a significant role in the pain associated hyperalgesic response.

Other factors have been found that may alter nitric oxide production and the outcome of the normal response. A combined increase in bacterial LPS and the cytokine IFN-γ, which are inducers of Nitric oxide synthase 2A, can modulate nitric oxide production and also the redox status of the tissues (Hecker et al, 1996; Hothersall et al, 1997). Similarly, a combined exposure of superoxide and interleukin-1β (IL-1β) resulted in a 10-fold amplification of nitric oxide production (Beck et al, 1998). Grossie et al (1997) have reported that patients with tumours have an enhanced toxicity to bacterial toxins, and that bacterial endotoxaemia was significantly increased in rats with transplanted Ward colon tumours compared with control rats. In the tumour burdened rats, the nitric oxide response was greater and there was an increase in serum ALT levels along with an increase in ornithine levels, a component of the urea cycle that accumulates with increased nitric oxide production (Albina et al, 1990). Thus bacterial toxins, inducers of nitric oxide synthase 2A, appear to be able to induce enhanced toxicity in disease states where nitric oxide is already being produced by other stimuli, such as tumours and inflammatory events. Interestingly inhibition of the nitric oxide response results in attenuation of bacterial-associated toxaemic shock (Ruetten et al 1996).

Thus alterations associated with the redox potential and the ability to induce nitric oxide responses, appear to play a significant role in pain responses and may be enhanced by secondary bacterial toxicity.

3.2.3. Organochlorine Pesticides in Polysymptomatic Pain/Fatigue Patients.

Chlorinated hydrocarbons such as hexochlorobenzene (HCB) and dichlorodiphenyltrichloroethane (DDT, or its metabolite 1,1-dichloro-2,2-*bis*(*p*-chlorophenyl)ethane, DDE) are recalcitrant chemicals, which have been used extensively world wide for pest control since the early 1940's and have been reported to have oestrogen-like and anti-anabolic effects. A cohort of subjects with defined chronic fatigue syndrome had an increased incidence and mean concentration of total serum chlorinated hydrocarbons compared with age- and sex-matched controls (Dunstan et al, 1995). Dunstan et al (1996) reported that patients with high DDE levels had a reduced percentage lymphocytes and an increased number of neutrophils and total white cell count (Dunstan et al, 1996) suggesting that organochlorines such as DDE may predispose the host to increased infections. Dunstan et al (1995) found DDE in 100% of subjects tested which is consistent with the observations of Stellman et al (1998) that found DDE in all adipose and serum samples taken from humans with the adipose tissues having much greater tissue levels. Stellman et al (1998) also found that serum and adipose tissue DDE levels were significantly correlated and they concluded that either serum or adipose tissue levels can be used as a measure of the body burden of the pesticide. Kreiss et al (1981) found that the total DDT and DDE levels were not associated with any specific illness or ill health, however, total DDT and DDE levels were positively associated with levels of serum cholesterol, triglyceride, and gamma-glutamyl transpeptidase (Kreiss et al, 1981). Ando & Wakisaka (1975) have previously reported the positive association between retained DDE levels and triglyceride levels in the serum and liver.

DDE is an anti-androgenic compound as it inhibits androgen binding to the androgen receptor (Kelce et al, 1995; Kelce et al, 1997; Sohoni & Sumpter, 1998). DDE was also found to inhibit Na^+K^+ATPase activity and glucose and L-tyrosine transport in rats (Iturri et al, 1989; Iturri & Wolff, 1982). Interestingly DDE has also been associated with a reduction in dopamine and noradrenaline levels in doves (Heinz et al, 1980), a reduction in brain γ-amino-butyric acid (GABA) and kidney tyrosine, proline and valine suggesting a sodium mediated alteration in amino acid transport (Dikshith et al, 1975). Also of importance, Ferreira et al (1997) reported that DDE interacted with mitochondrial complex II (succinate dehydrogenase) and results in a reduction in oxidative phosphorylation and membrane potential. These data suggest that organochlorine especially DDT and DDE may alter fatigue and the nitric oxide associated pain mechanisms.

Another organochlorine pesticide, Dieldrin, binds to both gamma-aminobutyric acid (GABA), the major inhibitory amino acid in the central nervous system, and monoamine receptors (Brannen et al, 1998; Sanchez-Ramos et al, 1998). Alterations in the GABA and monoamine receptors were most prominent in the brain stem, where the trigeminal nerve nuclei are found (Brannen et al, 1998). These data suggest that Dieldrin may also have éffects upon TMD symptom expression.

Due to the potential oestrogen-like, anti-anabolic influences and the induced changes in neurotransmitter activity of organochlorine pesticides and the increased female: male ratio for TMD an examination of the associations between organochlorine pesticides and TMD is warranted.

3.2.4. Alteration of Amino Acids in Polysymptomatic Pain/Fatigue Patients.

Total serum amino acid levels, particularly proline, serine, tryptophan and histidine were reduced in a cohort of patients with fibromyalgia (Russel et al, 1989), whilst reductions in serine and histidine and a potential anomaly in tryptophan transport have also been reported (Yunus et al, 1991). Patients with CFS have alterations in urinary excretion of amino and organic acids (McGregor et al, 1996a, 1996b, 1996c). Unknown molecule CFSUM1, together with tyrosine, β-alanine, aconitic acid and succinic acid were increased, whilst serine, alanine and glutamic acid were reduced compared with control subjects. These changes in urinary excretion patterns suggest an alteration in biochemical homeostasis involving enhanced protein degradation or proteolysis in patients with CFS. Furthermore, CFS patients with increased pain severity on a visual analogue scale had increases in urinary excretion of aspartic acid and CFSUM1, and reductions in serine and phenylacetic acid. Aspartic acid is a product of the AST reaction, but is also an excitatory amino acid that can activate the NMDA receptor associated hyperalgesic response as has been described in chronic pain (Coderre, 1993; Coderre & Yashpal, 1994; Thomas 1995). Additionally aspartic acid is a major amino acid substrate for the formation of argininosuccinate and arginine, the nitric oxide precursor (Figure 3.5) suggesting that increases in aspartic acid may enhanced the bodies ability to produce nitric oxide. These data provided evidence for a potential mechanism describing chronic pain *in vivo*, involving dysregulation of pain modulating neurotransmission and increased levels of excitatory amino acids leading to persistent nociceptive responses.

In fibromyalgia and CFS patients, the basal excretion of urinary catecholamines (Yunus et al, 1993) and the levels of hypothalamic-pituitary-adrenal axis neuropeptides were no different from controls (Cleare et al, 1995). However, patients with fibromyalgia and CFS had altered hypothalamic-pituitary hormonal excretion responses. Whilst fibromyalgia and CFS have been linked to depressive illness, the altered central nervous system (CNS) hormonal responses in patients with major depression, are distinct from those seen in CFS and fibromyalgia patients (Cleare et al, 1995; Moeller et al, 1994). It is now well accepted that CFS and fibromyalgia patients have dysregulation of prolactin and growth hormone excretion whilst major depressive patients have a dysregulated cortisol excretion (Cleare et al, 1995). Aguilar et al (1997) showed that prolactin secretion from the pituitary was associated with activation of NMDA receptor/nitric oxide/dopamine related mechanisms, which could also be inhibited by testosterone. Increased excitatory amino acid and nitric oxide levels, and reduced dopamine levels result in increased prolactin release. McCann et al (1998) discussed the evidence for alterations in the control of these hypothalamic/pituitary excretory mechanisms and its association with bacterial toxins, cytokines and nitric oxide production. The dysregulated prolactin/growth hormone secretion seen in chronic pain patients, is highly suggestive of a nitric oxide-dependent combined bacterial LPS/interleukin-1α,β (IL-1α, IL-1β) mediated mechanism, associated with a reduction in dopamine release (McCann et al, 1998). This is supported by the observation in fibromyalgia patients of reductions in serum cortisol, adrenaline and noradrenaline (Van Denderen et al, 1992).

No studies on TMD patients have been undertaken into these mechanisms, however data associating TMD with behavioural changes such as depression (Buckelew et al, 1986; Clark et al, 1985; Lee & Lee 1989; Pelz & Merskey 1982; Von Korff et al, 1988; Vimpari et al, 1995) have resulted in the psychophysiological hypothesis for TMD. Similarly TMD symptoms are associated with prescribed drugs that dysregulate dopamine metabolism, which also support this possibility, and is reviewed in Chapter 2 (Lynch et al, 1961; Malkin, 1964; Evans, 1965; Hiatt & Schwartz, 1966; Kraak, 1967; Pertoutka et al, 1988). In addition use of tricyclic anti-depressants (desipramine, nortriptyline, imipramine) that increase central nervous system noradrenaline availability and activity, have been used for pain relief in TMD patients (Tura & Tura, 1990). These data suggest that a reduction in noradrenaline and/or an increase in nitric oxide are likely to influence pain expression in TMD patients. The possible association between nitric oxide increases and TMD has been proposed by Anbar and Gatt (1988), and evidence of a possible increased nitric oxide response in TMD patients is provided by the reduction in mean blood pressure (de Abreu et al, 1993). Thus evaluation of amino acid metabolism in TMD patients is warranted.

3.2.5. Orofacial Pain Mechanisms.

Chronic pain phenomena are associated with NMDA associated hyperalgesia in both the CNS (Coderre, 1993; Coderre & Yashpal, 1994; Wong et al, 1998) and peripheral tissues (Holthusen & Arndt, 1995; Kang et al, 1995; Lorenzetti & Ferreira 1996). Coderre & Yashpal (1994) showed that activation of receptors or cellular mechanisms that increase the intracellular messengers, nitric oxide, arachidonic acid and protein kinase C lead to persistent nociception in response to tissue injury in rats. Hu et al (1993) and Yu et al (1995) revealed that jaw and neck muscle hyperactivity could be influenced by injection of mustard oil. Broton & Sessle (1988) and Broton et al (1988) showed that other algesic substances such as NaCl, KCl, bradykinins and histamine also activate the hyperalgesic process in the trigeminal system. Chiang et al (1997) showed that NMDA receptor activation was involved in the trigeminal nociceptive/hyperalgesic mechanism and that inhibition of the total receptor and the glycine receptor site inhibited the algesic response. Yu et al (1996) had previously shown that the NMDA receptor was involved in the development of increased jaw muscle electromyographic activity. Watanabe et al (1999) showed that antihistamines had an inhibitory effect upon palpable muscle tenderness in the head, neck and face. Thus alterations in nitric oxide, sodium, potassium and inflammatory mediators such as histamine may be involved in trigeminal nerve hyperalgesic responses, increased jaw muscle activity and pain.

3.2.6. Proteolysis (Intracellular Protein degradation).

The alterations in amino and organic acids in fibromyalgia and CFS patients may be the result of dysregulation of cellular protein turnover where there is a persistent increase in proteolysis over protein synthesis. This enhanced proteolysis is a mechanism that allows increased provision of amino acids from both muscle and liver following trauma, infection or highly stressful situations (Mortimore & Poso, 1987; Hasselgren, 1995). This mechanism has also been described in patients with certain genetic and acquired disease states, in muscle wasting associated with late stage cancer, as well as with cytokine action (Mortimore & Poso, 1987; Hasselgren, 1995).

Muscle proteolysis consists of two major mechanisms, the removal of: 1) short-term proteins (cytoplasmic proteins) or non-fibrillar proteolysis; and 2) long-term proteins (contractile proteins) or fibrillar proteolysis. In muscle, enhanced proteolysis can be related to both non-fibrillar (short-term proteins) and fibrillar (long-term protein - actin, myosin) mechanisms. Non-fibrillar proteolysis is associated with increased release of tyrosine from the cytoplasmic protein pool, whilst fibrillar proteolysis is associated with increased release of the unique actin component, 3-methylhistidine from muscle fibres (Hasselgren, 1995: Mortimore & Poso, 1987). Thus for muscle, these two processes can be assessed, using the release of tyrosine (non-fibrillar) and 3-methylhistidine (fibrillar), similar to that used to assess the actions of cytokines in proteolysis (Hasselgren, 1995). However the liver is also a major site of release of amino acids during sepsis, trauma and stressful events (Haussinger & vom Dahl, 1995). Liver and muscle, act as major control and storage sites for the release of amino acids during proteolytic events, and have different responses to cortisone, glucagon and noradrenaline (Hasselgren, 1995; Haussinger & vom Dahl, 1995). In sepsis, there is a generalised reduction in muscle amino acids as a result of reduced uptake of amino acids and increased efflux of amino acids from muscle tissue (Roth et al, 1982; Austgen et al, 1992). Conversely, in the liver, amino acid uptake is increased during sepsis (Ardawi, 1992; Austgen et al, 1991) and can be stimulated by both glucagon and noradrenaline (Canivet et al, 1980).

Muscle fibrillar and non-fibrillar proteolysis are two separate and independent processes (Mortimore & Poso, 1987). Muscle fibrillar proteolysis requires activation of calcium dependent proteases; whilst the non-fibrillar proteolytic response is controlled by insulin, glucagon, growth hormone, variation in cell volume, cyclic AMP, heavy metals, cytokines, the redox potential as well as the variation in specific amino acids (Tischler 1980; Rinehart et al, 1982; Fagan et al, 1987; Mortimore & Poso, 1987; Haussinger et al, 1991; Jimenez Jimenez et al, 1991; Balavoine et al, 1993; Fryburg & Barrett, 1993; Simmons et al, 1994; Hasselgren, 1995). Control of proteolysis in the liver is associated with variations in amino acids such as leucine, tyrosine, phenylalanine, glutamine, proline, methionine, tryptophan and histidine as well as an unusual promotional effect from alanine (Haussinger & vom Dahl, 1995). At normal physiological levels, the inhibitory effects of leucine and the other amino acids on non-fibrillar proteolysis, are potentiated by alanine (Mortimore & Poso, 1987). The increase in alanine levels during early exercise is thus likely to inhibit proteolysis and enhance protein synthesis in the liver (Wagenmakers, 1998). The amino acid leucine controls non-fibrillar proteolysis in muscle and adipose tissue, and has a distinct influence over RNA degradation (Balavoine et al, 1993). Thus alterations in the homeostasis of the proteolysis controlling amino acids may potentiate a dysregulation of the control of non-fibrillar proteolysis and may also reflect differences in muscle and liver amino acid metabolism. The variation in the pattern of urinary and serum amino acids are likely to reflect the underlying mechanisms associated with proteolysis and symptom expression.

Proteolysis and protein synthesis are closely associated with alterations in the redox potential and the ratio of oxidised nicotinamide adenine dinucleotide (NAD^+) and reduced nicotinamide adenine dinucleotide (NADH) (Tischler 1980; Rinehart et al, 1982; Fagan et al, 1987). Fagan et al (1987) showed that the induction of diabetes resulted in an alteration in the NADH: NAD^+ ratio and proteolysis, and suggested that alterations in insulin levels may play a major role in regulation of these processes. Hedden & Buse (1982) showed that whilst most NADH is derived from glycolysis, that degradation of the branched chain amino acids, leucine and isoleucine, will also result in increased NADH production and alteration in the NADH: NAD^+ ratio. Similarly the other proteolysis/protein synthesis associated hormones (glucagon, prolactin, growth hormone) also alter the NADH: NAD^+ ratio (Sistare & Haynes, 1985) and are associated with altered uric acid excretion (Yamamoto et al, 1996). These data indicate that the control of proteolysis exerted by leucine and the hormones such as insulin and glucagon, may be the result of alteration in the NADH: NAD^+ ratio. It is therefore worthy to note that Oles (1977) reported that anomalous glucose tolerance was associated with TMD symptoms; and Aaflot & Bruunsgaard (1994) identified that increasing levels of uric acid had an increased odds ratio for the development of fibromyalgia.

3.3. METHODS.

3.3.1. RDC\TMD type 1a patients.

3.3.1.a. Patient and Control Selection.

Forty-six patients sequentially presenting for orofacial pain management, complaining of orofacial muscle pain were recruited as previously described in chapter 2. These patients were designated to be the RDC/TMD type 1a patient group. Patients had a positive response on a VAS of average pain intensity in the 2 weeks prior to consultation, the presence of palpable muscle pain in the reported pain areas and that the pain was present on greater than 50% of days during the three months immediately preceding consultation.

Forty-one age and sex matched control subjects were recruited including, relatives of the pain patients and unrelated subjects to ensure similar socio-economic and ethnic backgrounds, as previously described in chapter 2. Control subjects (C), were eligible for inclusion in the study if they had no pain response to a VAS in the 2 weeks prior to consultation, did not give a history of chronic pain and had not sort professional advice or treatment for chronic muscle pain in the previous 12 months as previously described in Chapter 2.

3.3.1.b. Questionnaires.

All subjects completed a CPRU questionnaire and a Hopkins Symptom Check List-90-Revised (SCL-90-R), as previously described in chapter 2. The symptom incidence index was calculated as the number of positive responses to 48 symptoms from within the CPRU as previously reported in chapter 2.

3.3.1.c. Confirmation of Muscle Pain.

Muscle pain was confirmed by palpation of superficial facial, neck and shoulder and muscle pain and symptom severity was assessed using a VAS and the SCL-90-R question responses (Questions 1, Q4, Q12, Q14, Q27, Q39, Q40, Q42, Q52, Q55, Q56, Q58, Q66) as previously described in chapter 2.

3.3.1.d. Urine Specimens and GC-MS Identification.

Each subject collected the first morning urine specimen on the day of the second visit. The urine was refrigerated (not frozen) and processed within twenty-four hours of collection.

10mL aliquots were centrifuged at 1,500g for five minutes at 4°C and 200μL aliquots were transferred to derivatization tubes and freeze dried for eighteen hours.

The freeze dried urine material was then reacted to form the N(O,S)-heptafluorobutyryl-isobutyl (HFB-isobutyl) derivatives for analysis by GC-MS (MacKenzie & Tenaschuk, 1979a; 1979b). Two hundred microlitres of HCL-isobutanol were added to the freeze dried sample and heated at 110 °C for one hour. After cooling and evaporation by high purity nitrogen gas, the sample was freeze dried for thirty minutes. Ethyl acetate (50μL) and heptafluorobutyric anhydride (20 μL, HFBA) were added and the sample heated at 110 °C for thirty minutes. After cooling, the sample was evaporated with nitrogen gas and freezes dried for five minutes and then dissolved in 200μL of ethyl acetate. Derivatized urinary metabolites were separated using a Hewlett Packard 5890 Series II gas chromatograph (GC) and detected by a Hewlett Packard 5971A mass selective detector (MSD) as described previously (Walsh et al, 1995). Quality control was achieved by running a selected sample source every week with each batch of analyses to monitor extraction efficiency and machine response, and quantitative responses were assessed by an external standard strategy. Internal standard strategies were not used because these standards may co-elute with novel disease related metabolites in the chromatogram. Thirty-six urine peaks were selected for examination after confirming a linear detector response by the mass spectrometer. This is an extended list of metabolites from that used in the previous studies (McGregor et al, 1996a). Where possible, the peaks were identified by HP-UX Chemstation computer search of user-generated reference libraries (incorporating retention indices and mass spectra) and the WILEY Database™. The original peaks were numbered one to 28 in order of retention time and those peaks that could not be identified were allocated a reference code (either UM or CFSUM) as previously described (McGregor et al, 1996a). The additional peaks were allocated an alphabetic suffix (e.g., UM13a, UM15a) to conform to the retention time peak allocation method previously described (McGregor et al, 1996a). The area of each peak was recorded and percentage abundance of the 36 peaks was calculated for further analysis. The relative abundance analysis was included to allow assessment of metabolite changes, which were independent of variation in urine volume and total metabolite excretion.

Relative abundance or qualitative analyses of urine metabolites were performed using percentage analyses as used in previous studies (McGregor et al, 1996a; 1996b, 1996c). Quantitative urine analyses usually involve obtaining 24-hour urine samples. However, this study aimed to minimise dietary influence and standardise collection procedures, as well as maintain consistency with previous investigations, which have shown the value of this approach (McGregor et al, 1996a; 1996b, 1996c). The data were reviewed to assess possible quantitative differences and associations, which may be apparent from these types of urine analyses. Since 10 of the 36 measured urinary metabolites were unidentified components, the data were processed as GC-MS arbitrary peak area response units/μL, for statistical evaluation of group differences and associations.

3.3.2. CFS Group.

3.3.2.a. Study Population.

These data were collected as part of a study on CFS patients and not specifically directed at the TMD symptoms. This thesis reports on the association between reported TMD symptoms and biochemistry within this CFS population.

CFS patients and age and sex matched control subjects were recruited over an 18-month period, September 1994 - February 1996. The calculated study group sizes were required to be a minimum of 80 subjects per arm. The CFS patients were recruited from sequential medical practitioner referrals to the CFS clinical group at Royal North Shore Hospital from within the Sydney metropolitan area, and from advertisement in an ME/CFS newsletter. Two hundred and ten potential CFS subjects expressed an interest in participating in the study, of whom 114 agreed to participate and met the Centres for Disease Control (CDC) criteria (Holmes et al, 1988). Defined CFS patients were excluded at any time if they were given any alternative diagnosis or did not comply with additional study requirements.

The age- and sex-matched control subjects were recruited from two sources; friends and relatives of the CFS patients; and via advertisement of project patient recruitment at the University of Sydney and the University of Technology (Sydney). These two groups were selected to ascertain any differences between control subjects with exposure to CFS patients and control subjects with no exposure to CFS patients. One hundred and eight control subjects were assessed and excluded if they reported fatigue that influenced their lives or had evidence of any diagnosable illness or psychiatric disorder. Eighty-six control subjects were recruited.

All study participants were requested to cease the use of any alternative medicine or vitamin supplement for one week prior to sample collection. On the day of clinical examination and sample collection subjects were clinically assessed by either of 2 clinicians (PC-B, GF), completed specifically designed questionnaires, the John Hopkins Symptom

Checklist-90-Revised (SCL-90-R) (Degrotis, 1975) and had blood, microbiological swabs of nose and throat, venous blood and urine samples collected. The specifically designed questionnaires addressed medical history, symptom prevalence and severity, onset and duration of illness, treatments used, social demographics and other factors which may influence their illness (Appendix).

TMD symptoms were assessed clinically as presence or absence of facial pain and from scalar responses in a questionnaire to 12-month frequency and severity and 7 day severity to facial pain and TMJ pain. The questionnaire assessed the scalar severity response to facial and TMJ pain within the last 7 days and the last 12 months as well as a scalar response of the frequency of facial or TMJ pain over the last 12 months. No TMD clinical signs could be elicited in this study group as the study design explicitly excluded the author from any clinical involvement to ensure objectivity.

3.3.2.b. Urine Specimens and GCMS Compound Identification.

The collection of urine varied from that in the RDC/TMD type 1a patients. Each study subject recorded the time of the last urination during the evening prior to the day of sampling. All urine voided from the last voiding to the following appointment was collected into a 1L sterile container and the time of the last voiding recorded. The urine samples were coded, refrigerated (not frozen) at the collection point and transported to the laboratory for processing within twenty-four hours of collection as described in section 3.3.1.4. The excretion rate per minute was calculated by multiplying the peak area per ml of urine by the number of mls of urine excreted per minute and dividing the total by 1000. Thus 3 different urine data sets were available for analysis (excretion rate per minute, percentage excretion rate and absolute amount excreted) in the CFS cohort compared with 2 in the TMD/RDC type 1a cohort.

3.3.2.c. Blood Sample Collection and Analyses.

A 10mL blood specimen was collected in the early morning from overnight fasted subjects by venepuncture into glass EDTA vacutainers. Blood samples were coded stored at 4^{0}C and blood parameters assayed within 24 hours. The standard full blood count and serum chemistry was performed at the Royal North Shore Hospital Pathology Laboratories using a Coulter STKS (Coulter Electronic, Sydney, Australia). Blood samples collected in Becton Dickinson lithium heparin vacutainers, at the Royal North Shore Hospital, were stored at 4^{0}C and transported to the University of Newcastle. Samples were stored on ice during transportation, which occurred within 3-6 hours. On arrival at the University of Newcastle the plasma component of the whole blood samples was separated by centrifugation at 3000 rpm for 25 minutes at 4^0C (Beckman GS-15R centrifuge). All samples were processed within 8 hours of collection.

3.3.2.d. Extraction of Amino and Organic acids from Plasma.

Lipophilic components were extracted from the plasma, using methanol, to precipitate proteinaceous material, and a mixture of hexane and diethyl ether. The sample was vortexed for 60 seconds and centrifuged at 3000 rpm for 10 minutes at 4^0C. The upper hexane/ether phase was removed by Pasteur pipette and discarded. Following lipid extraction, the sample was again vortexed for 60 seconds and centrifuged at 3000 rpm for 20 minutes at 4^0C using methanol extraction. 2mL aliquots were transferred to a clean derivatization tubes and an internal standard (50μL pipecolic acid - 1nmol in distilled water) was added. Samples were snap frozen using liquid nitrogen and freeze-dried overnight, with the remaining alcohol removed by lyophilization.

HFB derivatization of the freeze-dried sample occurred as per section 3.3.1.4. Twenty-five plasma peaks were selected for evaluation. Where possible chromatographic peaks were identified by comparing the mass fragmentation patterns of each component together with its retention index to those held in mass spectral data libraries (user-generated reference libraries incorporating retention indices and mass spectra and the WILEY Database™) by HP-UX Chemstation computer search. Those peaks that could not be identified were allocated a reference code, unknown ph1 to unknown ph4. The data were expressed as mean peak area per mL of plasma. Standard curves for the amino acids ethanolamine, serine, alanine, glycine, β-alanine, valine, threonine, leucine, isoleucine, proline, s-methylcysteine, asparagine, aspartic acid, phenylalanine, ornithine, glutamic acid, lysine, tyrosine, aconitic acid and tryptophan were produced.

3.3.2.e. Extraction of Pesticide residues from Plasma.

The pesticide residues were extracted from plasma by the methods described in Dunstan et al (1995). The following pesticides were assessed: Aldrin, α- β- γ-BHC, Clordane, DDT, DDE, Deildrin, Endrin-1, Endrin-2, Endrin aldehyde, Endrin ketones, Endo sulphate, HCB, Heptochlor, Heptochlor epoxide, methoxychlor and PCB. Of these pesticides γ-BHC, Clordane, DDT, Endrin-1, Endrin-2, Endrin aldehyde, Endrin ketones, Endo sulphate, Heptochlor epoxide, methoxychlor and PCB were not detected in any blood samples from the CFS or control subjects. Therefore the analysis was restricted to Aldrin, α-β-BHC, DDE, Deildrin, HCB and Heptochlor.

3.3.3. RNase-L CFS study.

3.3.3.a. Patient selection.

This data set formed the experimental group in this study for the BSc(Hon) degree by Ms Lee Metcalf with the express purpose of examining the differences between CFS patients and control subjects. The TMD data was not reported and allows analysis of the associations between sIL-2r, IL-6, RNase-L and TMD.

Forty-two CFS patients who complied with the Fukuda et al (1994) CFS definition were recruited from general medical practitioners in the lower Hunter River district, after all subjects had the necessary clinical assessment to exclude all other possible causes of fatigue. Fifty community-based age- and sex-matched healthy control subjects were recruited from the friends and associates of the CFS patients. Thus these control subjects were close contacts of CFS patients. University of Newcastle Ethics approval was obtained. Patients completed a questionnaire that obtained scalar responses (0-4) to 86 questions on physical, cognitive and psychological symptoms over the preceding 7 days. This questionnaire is used as a standard questionnaire for poly-symptomatic patient symptom assessment (Appendix 4).

3.3.3.b. Blood Sample Collection and Analyses.

A 10mL blood specimen was collected in the morning from study subjects (32 CFS and 31 controls) by venepuncture into glass EDTA vacutainers. Blood samples were coded, stored at 4°C and blood parameters transported to a commercial laboratory for assessment of RNase-L activity, and the levels of soluble IL-2 receptor (sIL-2r) and IL-6 (Melbourne Forensic Diagnostic Laboratories, Melbourne, Australia). The RNase-L activity was assessed as total RNase-L and was not assessed to allow distinction between the 37Kd RNase-L identified to be increased in CFS patients (Suhadolnik et al, 1997).

3.3.4. Statistical Analysis.

All percentage data were arcsine transformed before analysis and other data not having a normal distribution were log transformed for usage in logistic regression analysis. Subject characteristics and symptom incidence was assessed using chi-square probability and students t-tests. Correction for multiplicity occurred where necessary. Univariate group differences were assessed on untransformed data using the non-parametric Mann Whitney U test and Spearman rank correlation, and ANOVA. Group differences were further assessed using multivariate standard and forward-stepwise discriminate function analyses, Pearson product moment analyses on the transformed data (significance $P \leq 0.05$; NS = not statistically significant). These data were processed using Access97 ™ (Microsoft, Redmond, WA, USA), Excel97 ™ (Microsoft) and Statistica ™ (Ver. 5.1, Statsoft, Tulsa, OK, USA).

3.4. RESULTS.

3.4.1. RDC/TMD type 1a study.

3.4.1.a. Patient Details.

Of the 43 RDC/TMD type 1a patients and 40 control subjects previously reported (McGregor et al, 1996d), ten RDC/TMD type 1a patients and six control subjects were excluded as urine samples were either not obtained (n=12) or laboratory processing problems occurred (n=4). The characteristics of the RDC/TMD type 1a pain and control (C) groups were closely matched in relationship to: numbers (C = 34; RDC/TMD type 1a = 29), age (C = 34.97±12.93; RDC/TMD type 1a = 38.17±12.74), sex characteristics (% Female; C = 67.6%; RDC/TMD type 1a = 82.8%) or marital status (% Married; C = 47.1%; RDC/TMD type 1a = 48.3%) of the remaining study subjects.

3.4.1.b. Urinary Excretion Patterns.

The mean relative abundance values, the mean concentrations and the mmolar values for known urinary metabolites analysed in this study are summarised in Table 3.1. Forward stepwise discriminant function analyses of the total concentration (Peak area) and relative abundance (amount of metabolite as a proportion of that excreted) data, indicated that the RDC/TMD type 1a group had a different urinary excretion profile compared with the control group (Concentration model: Wilk's λ=0.483, F=12.225, P<0.0000; Relative abundance model: Wilk's λ=0.310, F=9.276, P<0.0000). Leucine excretion was lower in the RDC/TMD type 1a group (Table 3.1) compared with the control group, and was the primary factor discriminating between the 2 study groups (both P<0.00006). The other primary discriminant factors in the concentration analysis were UM27 (P<0.03) and UM15 (P<0.04), whilst in the relative abundance analysis, the other primary discriminant factors were tyrosine (P<0.008) and serine (P<0.0008). Eighteen of the 29 RDC/TMD type 1a patients, compared with 2 of the 34 controls, had no detectable leucine in the urine (P<0.00001).

Tyrosine was higher in relative abundance in the RDC/TMD type 1a group and the tyrosine: leucine ratio was elevated in the RDC/TMD type 1a patients compared with the control subjects (RDC/TMD type 1a=7.21±1.91, C=1.13±0.23; P<0.00004). Thus the tyrosine: leucine ratio, the marker ratio of dysregulation proteolysis: protein synthesis, was strongly associated with facial pain.

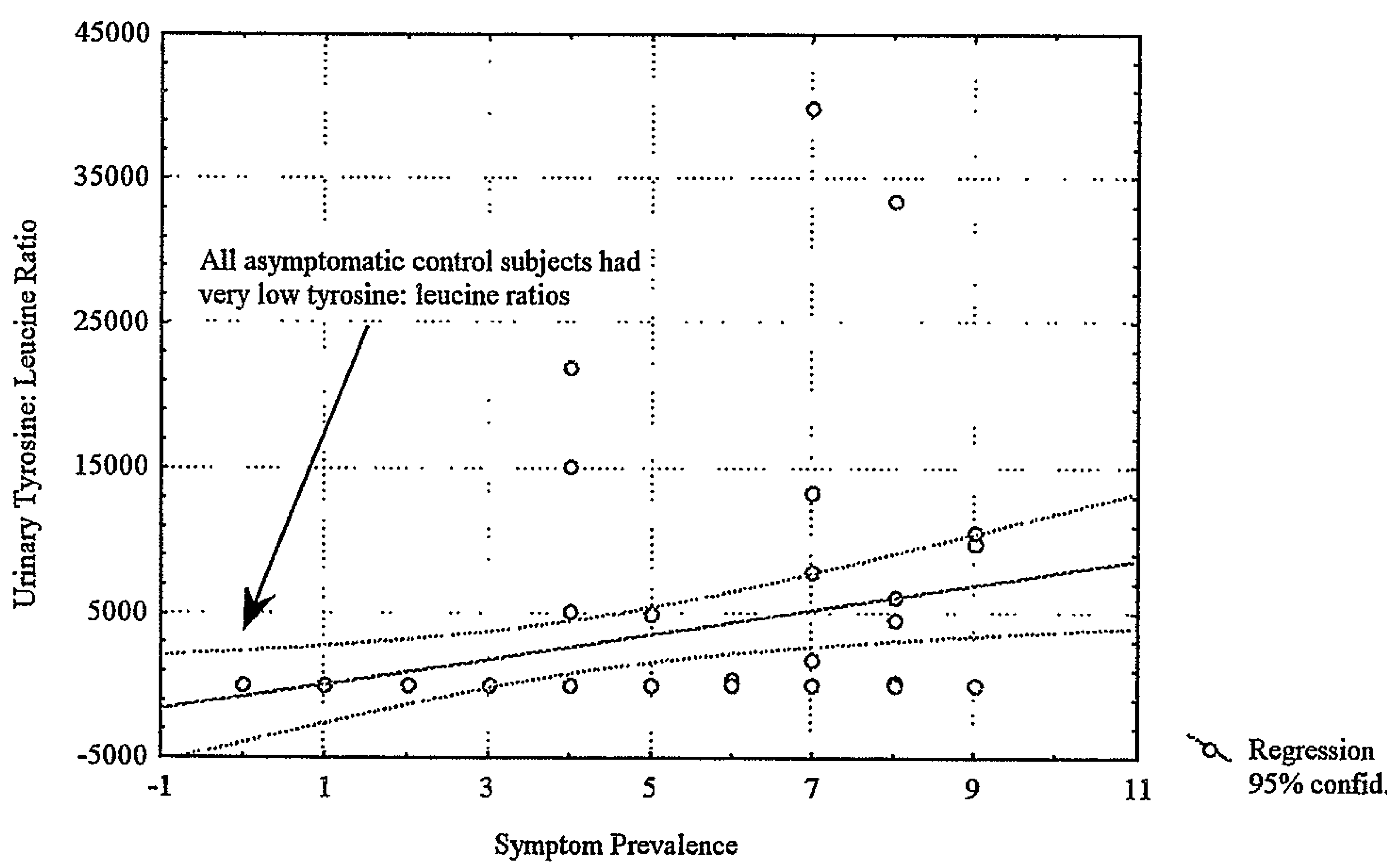
Table 3.1. T-test analysis of the Urine Excretion Data Measured in the RDC/TMD type 1a Patients and Control Subjects.

	Metabolite	Control		RDC/TMD type 1a		*P*	RA Control	RA MP	*P*
		Peak area(SE)	mmoles(SE)	Peak area(SE)	mmoles(SE)		%(SE)	%(SE)	
1	Ethanolamine	21.85(3.07)	2.70(0.03)	20.15(2.74)	2.69(0.03)	NS	1.68(0.15)	1.76(0.22)	NS
2	Serine	79.94(13.67)	0.87(0.05)	63.83(9.46)	0.81(0.03)	NS	7.21(0.50)	4.65(0.32)	$<2.0E^{-4}$
3	Alanine	55.84(7.97)	1.80(0.08)	41.77(6.14)	1.65(0.06)	NS	4.77(0.34)	3.25(0.37)	<0.003
4	Glycine	219.39(25.79)	1.98(0.11)	184.56(25.32)	1.84(0.10)	NS	19.64(1.43)	13.39(1.27)	<0.002
5	β-Alanine	7.69(1.82)	1.02(0.01)	11.30(2.52)	1.04(0.01)	NS	0.63(0.10)	0.86(0.15)	NS
5a	Valine	11.03(2.09)	0.12(0.01)	8.68(1.17)	0.11(0.00)	NS	0.95(0.08)	0.66(0.07)	<0.008
5b	Threonine	19.25(3.80)	1.23(0.01)	15.50(2.13)	1.22(0.01)	NS	1.64(0.14)	1.15(0.11)	<0.02
6	β-Aminoisobutyrate	5.99(1.23)	-	8.50(2.07)	-	NS	0.53(0.10)	0.70(0.16)	NS
7	Leucine	4.26(1.19)	0.11(0.00)	0.80(0.31)	0.10(0.00)	$<1.0E^{-6}$	0.32(0.04)	0.08(0.02)	$<1.0E^{-6}$
8	Phenylacetic acid	19.14(2.98)	-	23.07(4.35)	-	NS	1.83(0.27)	1.60(0.32)	NS
9	Proline	22.91(3.86)	0.59(0.01)	18.88(2.71)	0.58(0.01)	NS	2.01(0.20)	1.53(0.18)	NS
10	Succinic acid	15.85(2.66)	6.15(0.61)	21.63(4.52)	7.47(1.04)	NS	1.38(0.17)	1.45(0.29)	NS
10a	S-Methylcystiene	3.48(0.53)	0.80(0.01)	5.21(1.24)	0.83(0.02)	NS	0.31(0.06)	0.36(0.08)	NS
11	Asparagine	16.18(1.91)	0.17(0.02)	19.73(2.70)	0.21(0.03)	NS	1.66(0.22)	1.48(0.22)	NS
11a	Hydroxyproline	6.33(1.15)	0.72(0.00)	5.44(0.87)	0.72(0.00)	NS	0.48(0.05)	0.47(0.07)	NS
12	CFSUM1	30.92(7.52)	-	71.20(14.43)	-	<0.01	2.56(0.52)	4.73(1.07)	<0.03
13	UM13	0.54(0.19)	-	0.32(0.08)	-	NS	0.05(0.01)	0.03(0.01)	NS
13a	UM13a	1.71(0.41)	-	3.26(1.78)	-	NS	0.15(0.03)	0.24(0.11)	NS
14	UM14	1.47(0.49)	-	1.17(0.26)	-	NS	0.14(0.04)	0.09(0.02)	NS
15	UM15	0.15(0.08)	-	0.53(0.38)	-	NS	0.01(0.01)	0.03(0.01)	NS
15a	UM15a	5.12(0.83)	-	8.52(2.15)	-	NS	0.49(0.07)	0.73(0.18)	NS
15b	Acetaminophen	0.10(0.08)	-	6.08(3.36)	-	<0.03	0.01(0.01)	1.04(0.90)	NS
16	Aspartic acid	22.95(4.64)	0.22(0.02)	23.68(3.69)	0.23(0.01)	NS	1.87(0.12)	1.73(0.20)	NS
17	UM17	1.47(0.31)	-	1.83(0.50)	-	NS	0.13(0.03)	0.13(0.04)	NS
18	Phenylalanine	11.93(1.80)	0.41(0.00)	13.27(2.01)	0.41(0.00)	NS	0.94(0.07)	0.94(0.10)	NS
19	Ornithine	10.61(2.10)	1.56(0.00)	11.71(3.09)	1.56(0.00)	NS	0.78(0.11)	0.92(0.14)	NS
20	Glutamic acid	141.70(27.69)	0.99(0.10)	124.10(14.48)	0.93(0.05)	NS	11.86(0.86)	9.35(0.74)	<0.05
21	Lysine	58.07(16.99)	1.31(0.01)	55.56(16.96)	1.31(0.01)	NS	3.50(0.64)	4.27(1.02)	NS
22	Tyrosine	4.71(1.24)	2.87(0.00)	7.65(1.75)	2.87(0.00)	NS	0.32(0.06)	0.83(0.20)	<0.02
23	1-Methylhistidine	18.32(11.61)	0.32(0.01)	37.14(20.95)	0.33(0.01)	<0.03	0.54(0.31)	2.68(0.93)	<0.02
24	3-Methylhistidine	20.43(4.28)	0.02(0.01)	21.35(4.38)	0.03(0.01)	NS	1.73(0.23)	2.05(0.34)	NS
25	Hippuric acid	286.32(61.35)	1.46(0.27)	373.41(87.13)	1.83(0.38)	NS	20.14(2.58)	23.47(3.41)	NS
26	Aconitic acid	76.03(9.65)	0.43(0.03)	96.47(11.50)	0.48(0.03)	NS	7.01(0.77)	7.43(0.69)	NS
26a	Citric acid	24.59(11.76)	5.80(0.24)	50.57(21.23)	6.32(0.43)	NS	1.01(0.32)	2.92(0.79)	<0.04
27	UM27	23.44(9.49)	-	24.35(6.56)	-	NS	1.26(0.40)	2.13(0.38)	<0.04
28	UM28	13.72(8.26)	-	11.52(2.25)	-	$<5.3E^{-4}$	0.45(0.21)	0.87(0.15)	$<8.0E^{-4}$
	Total	1263.4(172.1)		1392.7(150.7)		NS			

Figure 3.6 shows the scatter plot of the association between the tyrosine: leucine ratio and the symptom prevalence (the cumulative total of the positive responses to the SCL-90-R pain marker questions - Q1-headaches, Q4-faintness and dizziness, Q12-chest pain, Q14-low in energy or run down, Q27-low back pain, Q39-heart palpitations, Q40-nausea, Q42-muscle soreness, Q52-numbness or tingling, Q55-trouble concentrating, Q56-weakness, Q58-heavy feelings in limbs, Q66-restless or disturbed sleep).

The control subjects who reported no symptoms (the asymptomatic group) had the lowest tyrosine: leucine ratio (ratio = 0.67±0.17 SD) followed by the symptomatic control group (ratio =1.26±0.29 SD) and the RDC/TMD type 1a patients (7.21±1.91 SD; ANOVA $P<0.0003$).

Figure 3.6. Scatter plot of the tyrosine: leucine ratio and its association with symptom prevalence in all RDC/TMD type 1a study subjects.



3.4.1.c. Differences in Urinary Metabolites in Relationship to Sex.

However forward stepwise discriminant function identified a difference in the urinary concentration excretion of amino and organic acids between males and females (Wilks' λ = 0.790, F=3.857, $P<0.008$). The first discriminate variable was leucine ($P<0.06$), followed by UM15a ($P<0.11$), β-alanine ($P<0.03$) and acetaminophen ($P<0.07$).

Forward stepwise discriminant function established a difference in the urinary concentration excretion of amino and organic acids between males and females (Wilks' λ = 0.610, F=2.667, $P<0.008$). The first discriminate variable was unknown UM17 ($P<0.13$), followed by leucine ($P<0.13$), glycine ($P<0.09$) and phenylacetic acid ($P<0.11$).

Student t-test did not find any difference in any urinary parameter (concentration or relative abundance). Thus the difference between males and females is not large but involve alteration in the metabolism of leucine, glycine, UM17, UM15a, β-alanine and phenylacetic acid, as well as increased consumption of acetaminophen.

3.4.1.d. Differences in Urinary Metabolites in Relationship to Pain Severity.

The RDC/TMD type 1a group had a mean VAS score of 2.8±1.0 (±SD), as distinct from the control group which had a zero VAS score. Multiple regression analysis was applied to determine whether the increases in pain severity scores observed in the RDC/TMD type 1a group were directly correlated with changes in the urine excretion profiles. Within the RDC/TMD type 1a group, the primary correlate with increasing pain severity scores in the regression models were the reductions in the concentration and relative abundance of excreted leucine (Table 3.2). Table 3.2 also shows a summary of both the concentration and relative abundance regression and correlation analyses of the urinary metabolites for all study subjects and specifically for the RDC/TMD type 1a patients.

Figure 3.7 shows the strong association between the tyrosine: leucine ratio and pain severity whilst Figure 3.8 shows the association between pain severity and total metabolite excretion. In the RDC/TMD type 1a group, leucine was the only urinary metabolite measured that decreased in output as the pain severity increased. Urinary excretion of 11 of the 36 (measured) metabolites had a positive association with pain severity score (Table 3.2), whereas the urinary concentrations of the remaining 25 metabolites were not correlated with the pain severity score. This was also seen for the mmolar concentrations of those amino acids assessed. The relative abundance of leucine was the only metabolite to correlate with changes in pain severity in the RDC/TMD type 1a group. Table 3.2 shows the increases in the excitatory amino acid, aspartic acid and glutamic acid, whilst Figure 3.9 shows the scatter plot of urinary aspartic acid concentrations in relationship to pain severity.

Thus the pain severity score was associated with alterations in the:

1) excretion of the non-fibrillar catabolism control amino acids (serine, glycine, valine, proline, phenylalanine, and glutamic acid), but not the fibrillar catabolism indicator, 3-methylhistidine;
2) tyrosine: leucine ratio suggesting dysregulation of proteolysis: protein synthesis;
3) total excretion of both amino and organic acids;

4) excretion of the phospholipid polar head-group amino acids, serine, ethanolamine; and

5) excretion of the excitatory amino acids, glutamic and aspartic acid.

These changes indicate an increase in both protein and phospholipid turnover with increasing pain severity.

Table 3.2. Summary of the regression and Spearman rank correlation analyses of the urinary excretion of metabolites with pain severity (VAS) scores in the RDC/TMD type 1a patients.

Metabolite	**Concentration**		**mmolar**		**Relative Abundance**	
All study subjects	r	*P*	r	*P*	r	*P*
Total amino acid excretion	0.43	<0.03				
Tyrosine: Leucine ratio	0.56	<0.002				
Increased						
UM28	0.66	$<1.0E^{-6}$	-	-	0.57	$<5.0E^{-5}$
UM27	0.54	<0.0002	-	-	0.44	<0.003
Tyrosine	0.46	<0.002	0.46	<0.002	0.33	<0.03
β-Alanine	0.42	<0.005	0.42	<0.004	0.22	NS
Citric acid	0.42	<0.005	0.42	<0.005	0.32	<0.04
CFSUM1	0.42	<0.005	-	-	0.25	NS
Aspartic acid	0.40	<0.007	0.40	<0.007	-0.01	NS
1-Methylhistidine	0.39	<0.008	0.39	<0.008	0.13	NS
Ethanolamine	0.38	<0.01	0.38	<0.01	0.13	NS
Phenylalanine	0.37	<0.02	0.37	<0.02	0.10	NS
Glutamic acid	0.35	<0.02	0.35	<0.02	-0.07	NS
β-Aminoisobutyrate	0.35	<0.02	-	-	0.14	NS
UM15a	0.30	<0.05	-	-	0.05	NS
Decreased						
Leucine	-0.63	$<3.0E^{-6}$	-0.63	$<3.0E^{-6}$	-0.68	$<3.0E^{-7}$
Glycine	0.10	NS	0.10	NS	-0.40	<0.007
Serine	0.25	NS	0.25	NS	-0.31	<0.05
MP patients only						
Total amino acid excretion	0.43	<0.03				
Tyrosine: Leucine ratio	0.56	<0.002				
Increased						
Aspartic acid	0.55	<0.003	0.55	<0.002	0.34	NS
Glutamic acid	0.55	<0.003	0.55	<0.002	0.31	NS
Serine	0.49	<0.009	0.49	<0.009	0.29	NS
Ethanolamine	0.48	<0.01	0.48	<0.01	0.15	NS
Aconitic acid	0.43	<0.03	0.43	<0.03	0.17	NS
UM15a	0.42	<0.03	-	-	0.13	NS
Valine	0.40	<0.04	0.40	<0.04	0.29	NS
Phenylalanine	0.40	<0.04	0.40	<0.04	0.22	NS
UM17	0.39	<0.04	-	-	0.27	NS
Proline	0.38	<0.05	0.38	<0.05	0.11	NS
Glycine	0.38	<0.05	0.38	<0.05	0.05	NS
Decreased						
Leucine	-0.49	<0.008	-0.49	<0.008	-0.47	<0.02

Concentration regression model: R^2=0.999, F=4705, P<0.02,
Variables: 1) aspartic acid +P<0.02, 2) leucine -P<0.009, 3) acetaminophen +P<0.03.
Relative abundance, regression model: R^2=0.848, F=4.450, P<0.007,
Variables: 1) leucine -P<0.0002, 2) serine -P<0.95, 3) citric acid +P<0.02.

The mmolar and concentration values were identical and will not shown for subsequent analyses.

Figure 3.7 Scatter plot of the tyrosine: leucine ratio and its association with pain severity in all RDC/TMD type 1a study patients.

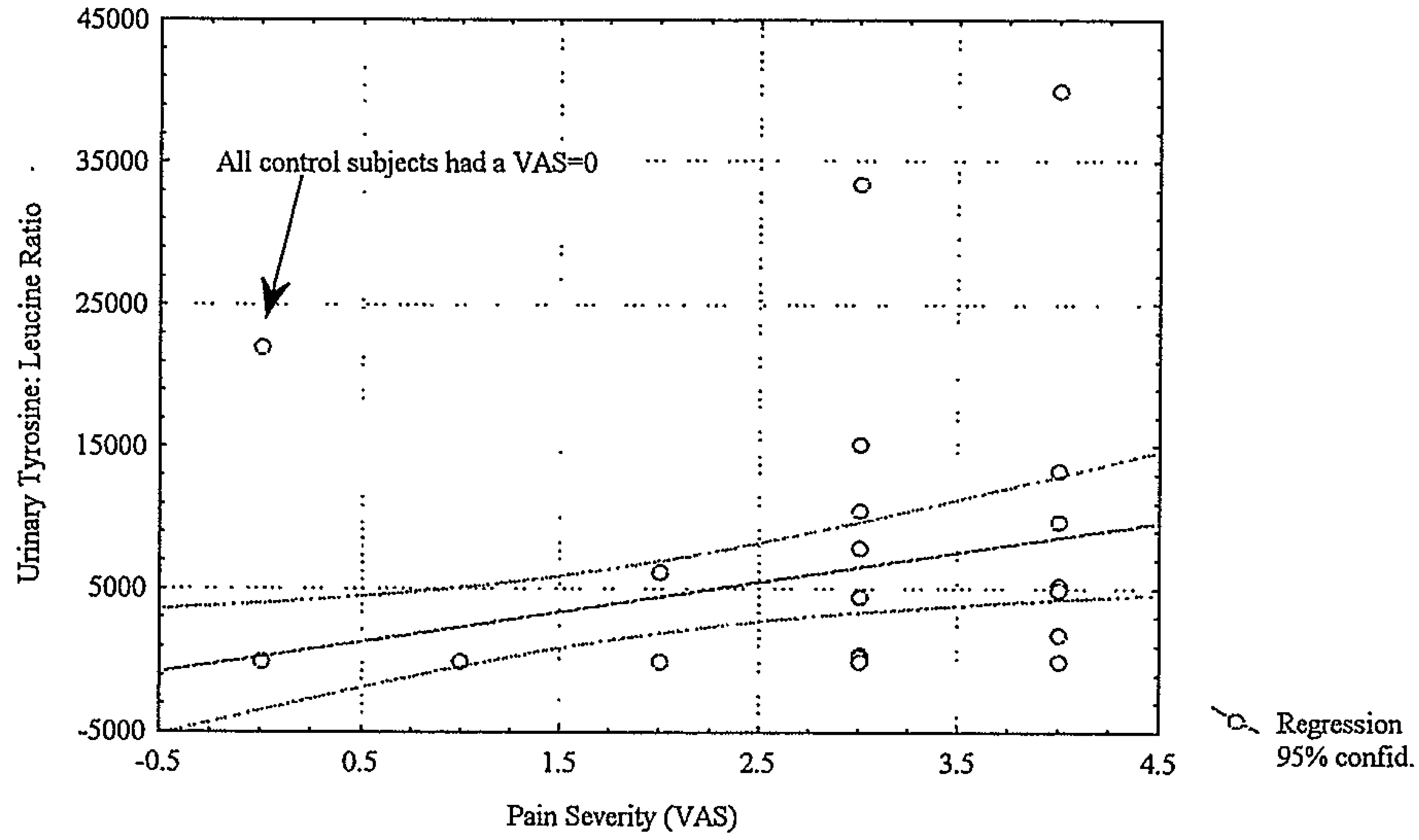


Figure 3.8 Scatter plot of the tyrosine: leucine ratio and its association with the total metabolite excreted in all RDC/TMD type 1a study patients.

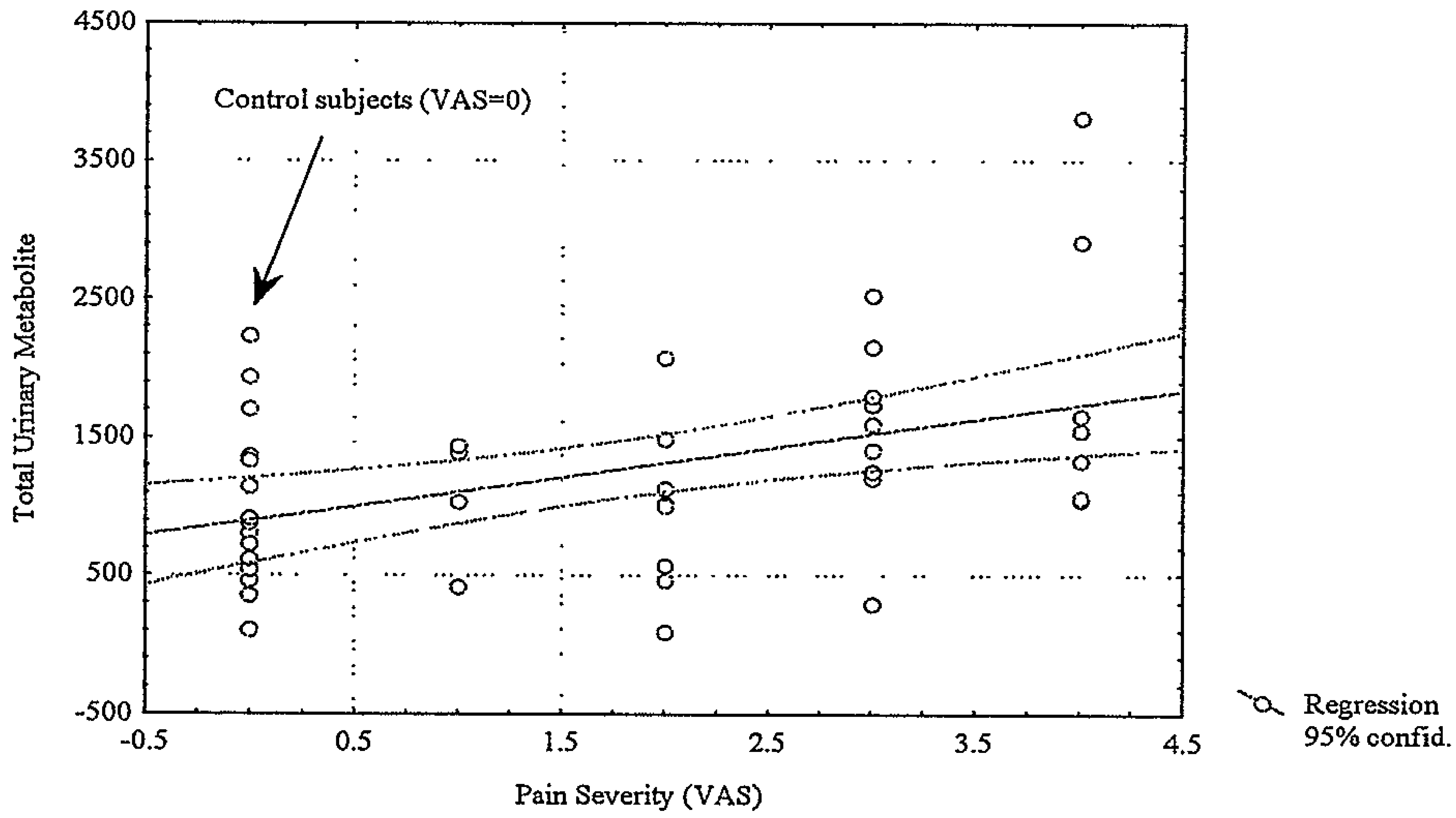
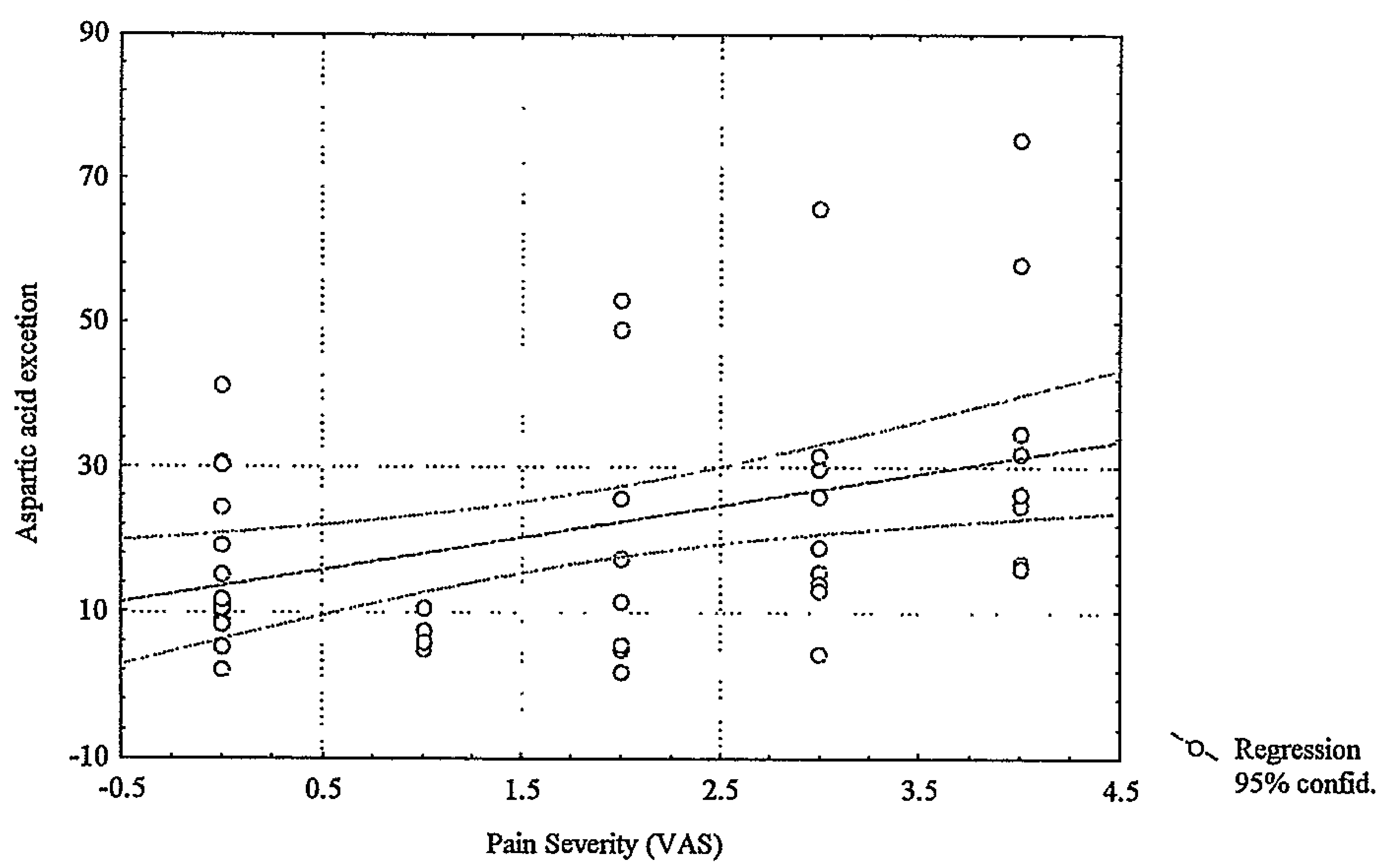


Figure 3.9. Scatter plot of pain severity and its association with aspartic acid excretion in the RDC/TMD type 1a study subjects.



3.4.1.e. Differences in Urinary Metabolites in Relationship to Symptom Prevalence.

The RDC/TMD type 1a group had an increase in the reporting of pain/fatigue symptoms (C = 2.1±1.7; RDC/TMD = 6.6±2.0 – $P<2.0E^{-12}$). Multiple regression analysis was applied to determine whether the increases in symptom prevalence were directly correlated with changes in urine excretion profiles. Within the RDC/TMD type 1a group, the primary correlate with increasing symptom prevalence, was the reduction in concentration and relative abundance of leucine (Table 3.3). Table 3.3 also shows a summary of the concentration and relative abundance regression and correlation analyses of the urinary metabolites of all study subjects and within the RDC/TMD type 1a patients. The association between the tyrosine: leucine ratio and symptom prevalence is shown in Figure 3.6 and was similar to that seen with pain severity.

Table 3.3. Summary of the regression and Spearman rank correlation analyses of the urinary excretion of metabolites with symptom prevalence in the RDC/TMD type 1a patients.

Metabolite	**Concentration**		**Relative Abundance**	
All study subjects	r	*P*	r	*P*
Tyrosine: leucine ratio	0.33	<0.008		
Total amino acid excretion	0.12	NS		
Increased				
UM28	0.43	<0.001	0.37	<0.003
Acetaminophen	0.38	<0.002	0.30	<0.02
CFSUM1	0.27	<0.04	0.22	NS
Tyrosine	0.20	NS	0.27	<0.04
Decreased				
Leucine	-0.53	$<7.0E^{-6}$	-0.58	$<4.0E^{-7}$
Alanine	-0.19	NS	-0.39	<0.002
Glycine	-0.12	NS	-0.37	<0.003
Serine	-0.09	NS	-0.37	<0.003
Valine	-0.14	NS	-0.35	<0.005
Threonine	-0.08	NS	-0.29	<0.03
Glutamic acid	-0.03	NS	-0.27	<0.04
MP patients only				
Glutamic acid	-0.13	NS	-0.37	<0.05

Concentration regression model: R^2=0.626, F=5.742, $P<0.00000$
Variables: 1) leucine -$P<0.00001$, 2) S-Methylcysteine +$P<0.002$, 3) Acetaminophen +$P<0.005$.
Relative abundance, regression model: R^2=0.673, F=8.570, $P<0.00000$
Variables: 1) leucine -$P<0.000001$, 2) Acetaminophen +$P<0.008$, 3) S-Methylcysteine +$P<0.02$.

Thus symptom severity was associated with alterations in the:

1) excretion of the non-fibrillar catabolism control amino acids (serine, glycine, valine, leucine, and glutamic acid);
2) tyrosine: leucine ratio suggesting dysregulation of proteolysis: protein synthesis; and
3) the muscle liver nitrogen exchange amino acids, alanine and glutamic acid.

These changes indicate an increase in both protein turnover and reductions in the availability of muscle produced nitrogen transfer amino acids.

3.4.1.f. Differences in Urinary Metabolites in Relationship to the Duration of Illness.

The RDC/TMD type 1a group was assessed by multiple regression analysis to determine whether the increases in illness duration were directly correlated with changes in the urine excretion profiles. Within the RDC/TMD type 1a group, the primary correlate with increasing illness duration (in the concentration regression model and the correlation assessment) was the reduction in succinic acid levels (Table 3.4). The association between succinic acid and total metabolite excreted with duration of the pain illness is shown in Figures 3.10 and 3.11. The regression analysis for the relative abundance data found that 3-methylhistidine was the prime discriminate variable; this was reflected in the increase seen in Table 3.4.

Table 3.4. Summary of the regression and Spearman rank correlation analyses of the urinary excretion of metabolites with duration in the RDC/TMD type 1a patients only.

Metabolite	**Concentration**		**Relative Abundance**	
MP patients only	r	P	r	P
Total metabolite excreted	-0.52	<0.009		
Increased				
3-Methylhistidine	0.26	NS	0.59	<0.003
UM27	0.05	NS	0.42	<0.05
Decreased				
Succinic acid	-0.58	<0.003	-0.44	<0.04
Hippuric acid	-0.52	<0.01	-0.39	NS
Serine	-0.46	<0.03	-0.08	NS
Glutamic acid	-0.46	<0.03	-0.02	NS
Phenylacetic acid	-0.45	<0.03	-0.30	NS
Asparagine	-0.42	<0.05	-0.12	NS
Aspartic acid	-0.41	<0.05	-0.02	NS
Concentration regression model: R^2=0.879, F=9.486, P<0.0002				
Variables: 1) succinic acid -P<0.006, 2) leucine -P<0.09, 3) UM17 +P<0.11				
Relative abundance regression model: R^2=0.999, F=584600, P<0.001				
Variables: 1) 3-methylhistidine +P<0.18, 2) aconitic acid -P<0.27, 3) UM13 +P<0.30				

The increase in duration of the illness was associated with quite different metabolite changes to that seen with pain severity and symptom prevalence. Illness duration was negatively associated with total amino acid excretion. In the RDC/TMD type 1a group, the relative abundance of 3-methylhistidine was negatively correlated with the total metabolite (r=-0.62, P<0.001), and positively correlated with the relative abundance of both leucine (r=-0.47, P<0.01) and tyrosine (r=0.46, P<0.01). However, in the control subjects, the relative abundance of 3-methylhistidine was not correlated with the total metabolite (r=-0.06) or the relative abundance of leucine (r=-0.11) or tyrosine (r=0.06).

Figure 3.10. Scatter plot of the urinary concentration of succinic acid in association with the duration of the illness in the RDC/TMD type 1a patients.

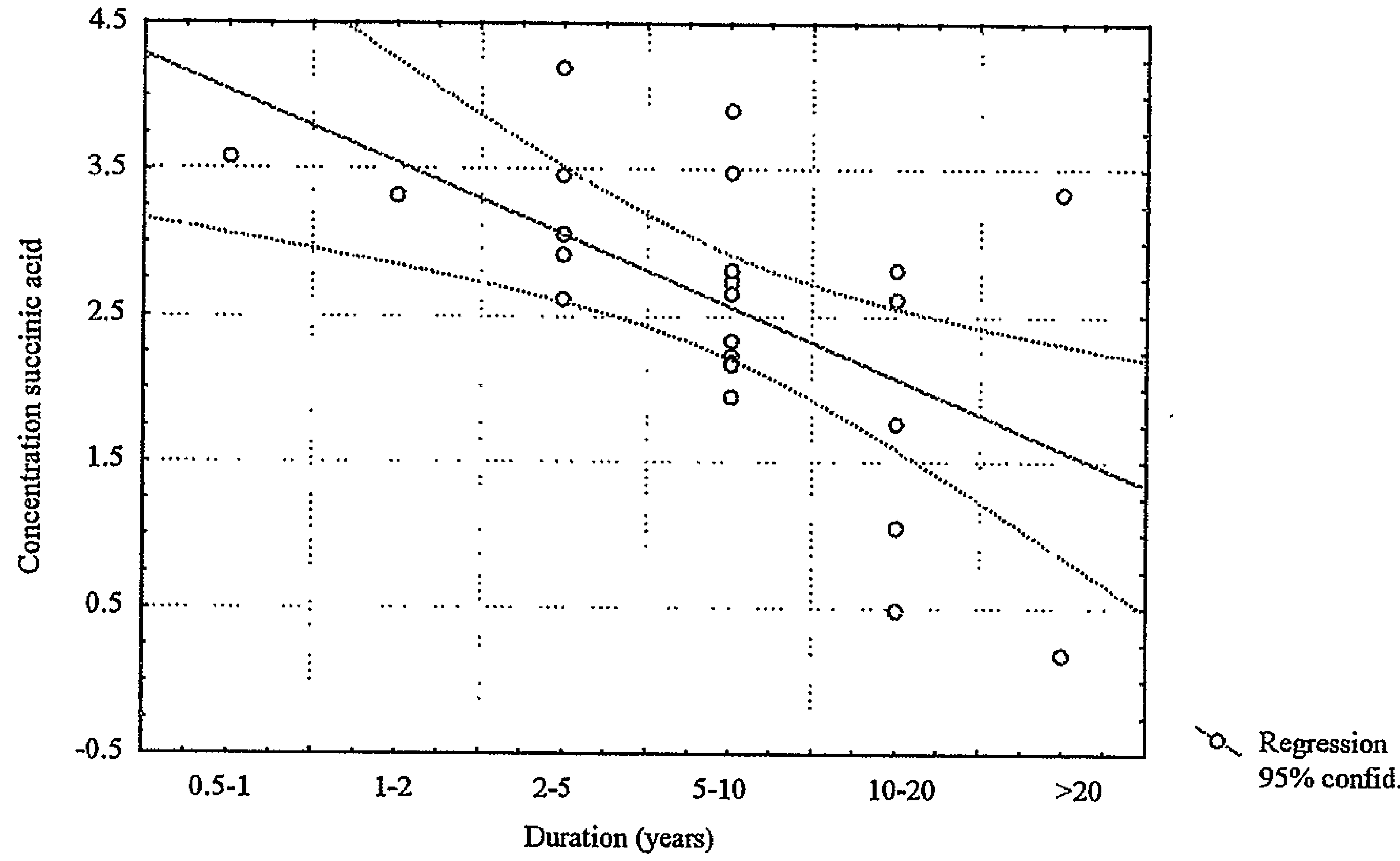
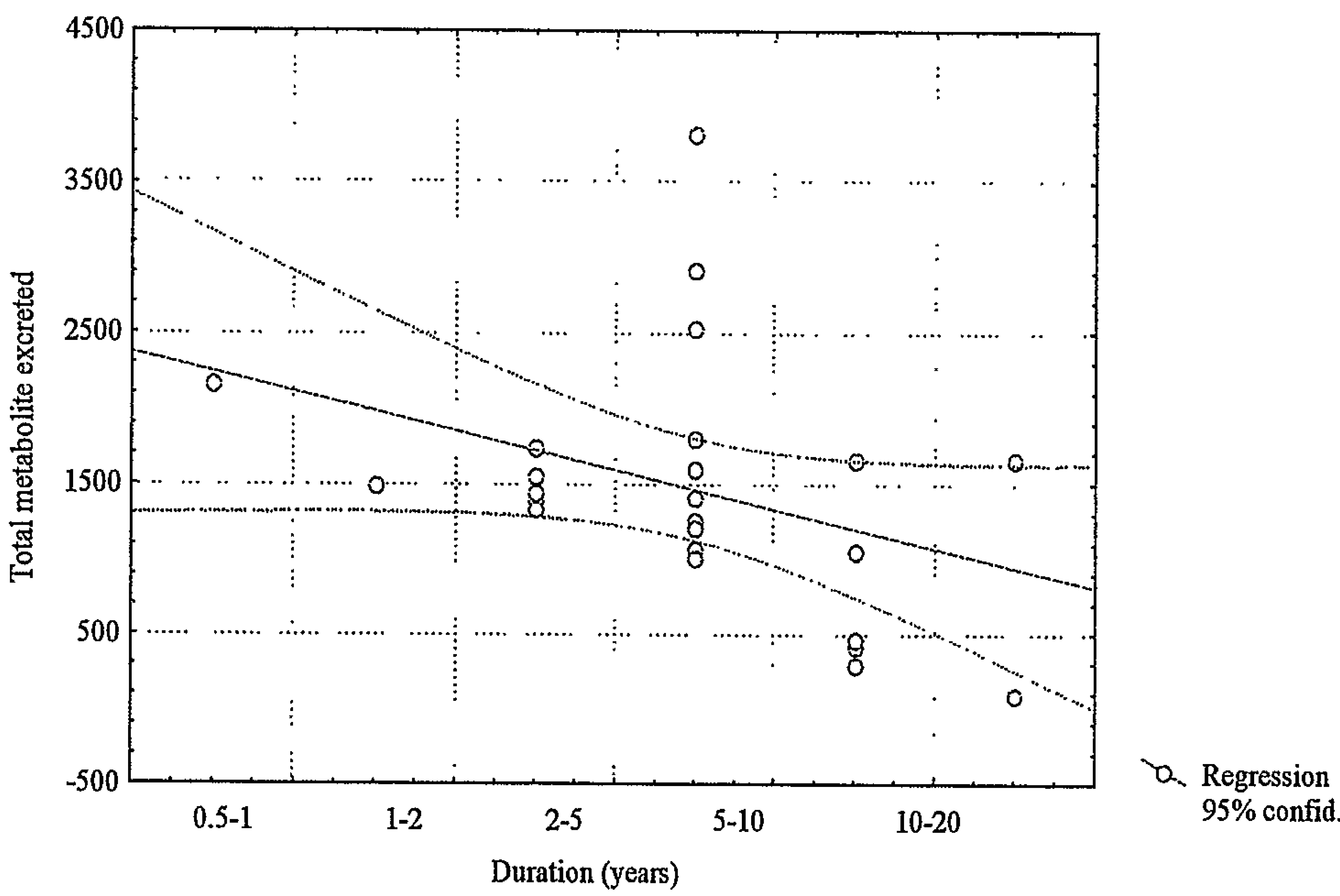


Figure 3.11. Scatter plot of the total metabolite excreted per RDC/TMD type 1a patient in relationship to the duration of TMD symptoms.



These data show that as the total amino acid excretion falls with increasing duration of the illness, there is a corresponding increase in the excretion of the fibrillar catabolism marker, 3-methylhistidine in the RDC/TMD type 1a group. This increase in 3-methylhistidine is associated with increases in the relative abundance of both tyrosine and leucine, indicating a corresponding increase in protein synthesis, non-fibrillar and fibrillar proteolysis. Thus with increasing illness duration there was a progressive reduction in amino acid excretion, which was negatively associated with increases in marker metabolites suggesting increases in fibrillar and non-fibrillar proteolysis and protein synthesis.

3.4.1.g. Differences in Urinary Metabolites in Relationship to Sudden or Gradual Onset.

The progressive reduction in total amino acid excretion associated with increasing illness duration and increased proteolytic activity offers the basis for the observed gradual onset in most TMD patients. Table 3.5 shows the multiple regression analyses of the differences between RDC/TMD type 1a pain patients with sudden and gradual onsets. Hydroxyproline was the principle difference between the sudden and gradual RDC/TMD type 1a pain patients. Table 3.5 also shows the summary of the t-test analysis. Hydroxyproline and UM13a were the two metabolites that were different in concentration and relative abundance analysis. These data show that RDC\TMD type 1a patients reporting a sudden onset can be differentiated from those reporting a gradual onset, on the basis of their urinary profiles. These changes are associated with increased excretion of the mitochondrial respiratory chain organic acid, succinic acid, and connective tissue and cartilage metabolite, hydroxyproline, rather than tyrosine, leucine or 3-methylhistidine. These data suggest dysregulation of oxidative phosphorylation and connective tissue turnover is more prominent in patients reporting a sudden onset.

Table 3.5. Summary of the t-test difference in the urinary excretion of metabolites related to a sudden or gradual onset in the RDC/TMD type 1a patients only.

Metabolite	**Concentration**			**Relative Abundance**		
	Sudden	Gradual	*P*	Sudden	Gradual	*P*
Total metabolite	1575±1009	1409±720	NS			
tyrosine: leucine ratio	6880±10702	5709±10840	NS			
Increased						
Hydroxyproline	9.19±5.69	4.23±3.19	<0.02	0.75±0.46	0.36±0.24	<0.01
UM13a	8.13±16.62	1.32±1.61	<0.05	0.58±1.02	0.10±0.13	<0.02
Decreased						
Succinic acid	11.5±9.9	22.2±17.5	NS	0.74±0.48	1.5±1.1	<0.04
Concentration regression model: R^2=0.990, F=27.358, P<0.0009 Variables: 1) Hydroxyproline +P<0.00003, 2) threonine -P<0.34, 3) 1-methylhistidine +P<0.0004						
Relative Abundance regression model: R^2=0.999, F=187.13, P<0.0006 Variables: 1) Hydroxyproline +P<0.00003, 2) β-aminoisobutyrate -P<0.07, 3) Lysine -P<0.0001						

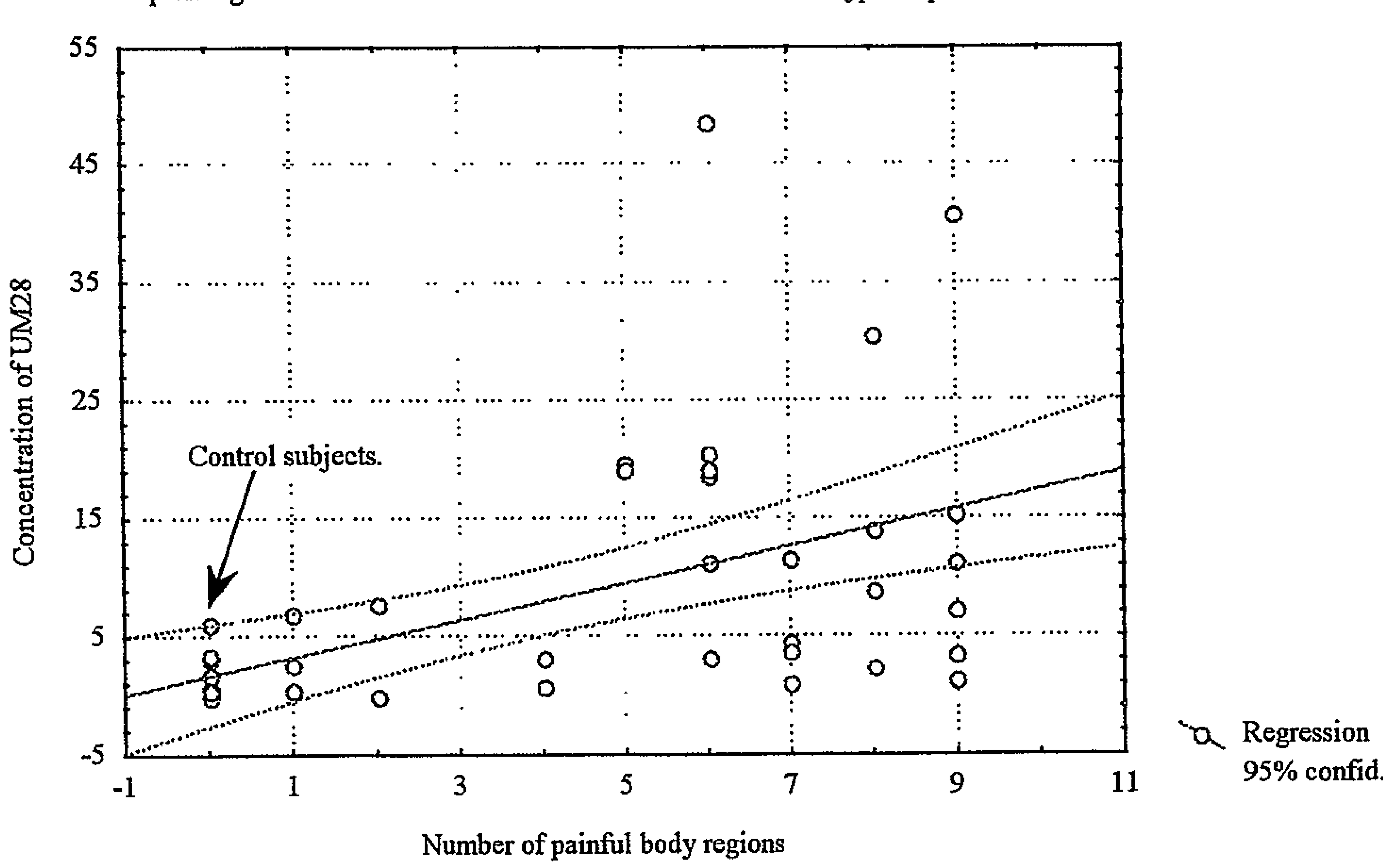
3.4.1.h. Differences in Urinary Metabolites in Relationship to Body Pain Distribution.

In the RDC/TMD type 1a group, increases in body pain distribution correlated positively with increases in duration (r=0.50, P<0.01) and symptom prevalence (r=0.61, P<0.0006) but not pain severity (r=0.32). Multiple regression analysis was applied to determine whether increases in the number of body pain regions (facial, head, neck, arm, anterior chest, arm, low back, abdomen, leg) observed in the RDC/TMD type 1a group, were directly correlated with changes in the urine excretion profiles (Table 3.6). The primary correlate with increasing body pain region in the regression models were increases in the concentration and relative abundance of excreted UM28 (Table 3.6 and Figure 3.12). Table 3.6 summarises both the concentration and relative abundance analyses of the urinary metabolites, for the entire group of study subjects. Within the RDC/TMD type 1a group, body pain distribution was not correlated with any metabolite.

Table 3.6. Summary of the regression and Spearman rank correlation analyses of the urinary excretion of metabolites with the body pain regions in the RDC/TMD type 1a patients.

Metabolite	Concentration		Relative Abundance	
All study subjects	r	*P*	r	*P*
Tyrosine: leucine ratio	0.60	<0.00001		
Total amino acid excretion	0.30	<0.05		
Increased				
UM28	0.64	<0.000002	0.63	<0.000004
1-Methylhistidine	0.45	<0.003	0.23	NS
UM27	0.43	<0.004	0.43	<0.004
CFSUM1	0.42	<0.005	0.32	<0.04
Citric acid	0.37	<0.02	0.31	<0.05
β-Alanine	0.32	<0.04	0.19	NS
Tyrosine	0.31	<0.04	0.25	NS
Decreased				
Leucine	-0.54	<0.0002	-0.58	<0.00003
Glycine	-0.06	NS	-0.47	<0.002
Valine	-0.01	NS	-0.29	<0.05
Serine	0.06	NS	-0.34	<0.03
Concentration regression model: R^2=0.844, F=14.413, *P*<0.00000				
Variables: 1) UM28 +*P*<0.000002, 2) phenylacetic acid -*P*<0.003, 3) leucine -*P*<0.12.				
Relative abundance regression model: R^2=0.804, F=12.308, *P*<0.00000				
Variables: 1) UM28 +*P*<0.000001, 2) 1-methylhistidine +*P*<0.005, 3) leucine -*P*<0.004.				

Figure 3.12. Scatter plot of the association between increasing number of body pain regions and the concentration of UM28 in RDC/TMD type 1a patients.



The prime discriminate variable for increasing body pain distribution (all subjects) was UM28 and was different from that of pain severity, however increasing body pain distribution was positively associated with the tyrosine: leucine ratio and total amino acids excreted as seen with increasing pain severity. Thus increasing body pain distribution is similar to pain severity when assessing all study subjects, but is not associated with any urinary amino or organic acid alteration within the RDC/TMD type 1a pain group.

3.4.1.i. Differences in Urinary Metabolites in Relationship to Muscle Pain/Soreness.

Within the RDC/TMD type 1a patients, muscle soreness as assessed by SCL-90-R question 42 (soreness of your muscles), was correlated with symptom prevalence (r=0.69, P<0.002), body pain distribution (r=0.65, P<0.007) and pain severity (r=0.56, P<0.03), but not the duration of the illness (r=0.34). Multiple regression analysis was applied to determine the associations with changes in the urine excretion profiles. Within the RDC/TMD type 1a group, the principle metabolites that correlated with increasing muscle soreness in the regression models, were the reduction in leucine and increase in UM28 (Table 3.7). Table 3.7 also summarises of both the concentration and relative abundance analyses of the urinary metabolites within the entire study subjects and within the RDC/TMD type 1a patients. Muscle soreness shows many similar urinary amino and organic acid changes to that seen with body pain distribution.

Table 3.7. Summary of the regression and Spearman rank correlation analyses of the urinary excretion of metabolites with muscle soreness scores in the RDC/TMD type 1a patients.

Metabolite	Concentration		Relative Abundance	
All study subjects	r	P	r	P
Tyrosine: Leucine ratio	0.35	<0.02		
Increased				
UM28	0.37	<0.009	0.45	<0.001
Citric acid	0.26	NS	0.31	<0.03
Decreased				
Leucine	-0.31	<0.03	-0.32	<0.03
Glycine	-0.17	NS	-0.32	<0.03
Serine	-0.18	NS	-0.32	<0.03
MP patients only				
Increased				
Citric acid	0.54	<0.03	0.68	<0.002
Decreased				
Glycine	-0.30	NS	-0.55	<0.03
Concentration regression model: R^2=0.649, F=3.883, P<0.0001 Variables: 1) leucine +P<0.002, 2) +UM28 -P<0.04, 3) UM15 -P<0.06. **Relative abundance** regression model: R^2=0.609, F=5.342, P<0.00001 Variables: 1) serine -P<0.0007, 2) aspartic acid +P<0.006, 3) acetaminophen +P<0.07				

3.4.1.j. Differences in Urinary Metabolites in Relationship to Fatigue.

Within the RDC/TMD type 1a patients, fatigue as assessed by SCL-90-R question 14 (feeling low in energy or run down), was correlated with symptom prevalence (r=0.72, P<0.002) and body pain distribution (r=0.60, P<0.02), but not pain severity (r=0.34) or the duration of the illness (r=0.19). Table 3.8 summarises the concentration and relative abundance multiple regression and correlation analyses of the urinary metabolites within the entire study group. Whilst leucine and 3-methylhistidine were the principle metabolites associated with fatigue when the whole study population was assessed, within the RDC/TMD type 1a group, the principle metabolites correlated with increasing fatigue were reductions in UM13a and succinic acid and increases in 3-methylhistidine.

Table 3.8. Summary of the regression and Spearman rank correlation analyses of the urinary excretion of metabolites with fatigue scores in the RDC/TMD type 1a patients.

Metabolite	**Concentration**		**Relative Abundance**	
All study subjects	r	P	r	P
Increased				
UM28	0.34	<0.02	0.35	<0.02
3-Methylhistidine	0.20	NS	0.35	<0.02
Tyrosine	0.23	NS	0.32	<0.03
UM27	0.20	NS	0.33	<0.03
Decreased				
Leucine	-0.41	<0.004	-0.39	<0.006
Succinic acid	-0.11	NS	-0.34	<0.02
Glycine	-0.11	NS	-0.33	<0.03
Glutamic acid	-0.05	NS	-0.30	<0.04
Phenylacetic acid	-0.12	NS	-0.29	<0.04
MP patients only				
Increased				
3-Methylhistidine	0.43	NS	0.52	<0.04
Decreased				
UM13a	-0.56	<0.02	-0.68	<0.003
Succinic acid	-0.45	NS	-0.50	<0.04
Concentration regression model: R^2=0.633, F=6.553, P<0.00001 Variables: 1) leucine -P<0.0003, 2) 3-methylhistidine P<0.0003, 3) UM14 P<0.005, **Relative abundance** regression model: R^2=0.707, F=7.237, P<0.00000 Variables: 1) leucine -P<0.00003, 2) phenylacetic acid -P<0.005, 3) 3-methylhistidine P<0.003.				

Fatigue was a primary symptom for determining the difference between fibromyalgia-like RDC\TMD type 1a patients and the remaining RDC\TMD type 1a patients, and was associated with increasing body pain distribution and symptom prevalence. These data show that whilst muscle soreness and fatigue are common symptoms in RDC/TMD type 1a patients, they are related to different metabolites, which shows that they are associated with different biochemical events.

3.4.1.k. Differences in Urinary Metabolites in Relationship to SCL-90-R Somatization.

Axis II of the RDC/TMD criteria requires the assessment of the SCL-90-R somatization dimension. Within the RDC/TMD type 1a patients, the SCL-90-R somatization dimension was correlated with pain severity (r=0.70, $P<0.003$) and symptom prevalence (r=0.62, $P<0.008$), but not with body pain distribution (r=0.44) or duration of the illness (r=0.30). Multiple regression analysis was applied to determine the associations between the SCL-90-R somatization dimension and the urine excretion profiles and is shown in Table 3.9. Table 3.9 also shows the summary of the correlation analyses. The principle metabolites associated with somatization were increases in 3-methylhistidine and reductions in glycine and leucine. No correlation was found between somatization and any metabolite within the RDC/TMD type 1a group. Thus somatization scores were associated with some of the biochemical changes noted in pain patients.

Table 3.9. Summary of the regression and Spearman rank correlation analyses of the urinary excretion of metabolites with SCL-90-R somatization and depression scores in the RDC/TMD type 1a patients.

Metabolite	Concentration		Relative Abundance	
Somatization Dimension				
All study subjects	r	*P*	r	*P*
Tyrosine: leucine ratio	0.37	<0.01		
Increased				
UM28	0.41	<0.004	0.39	<0.005
CFSUM1	0.32	<0.03	0.33	<0.03
Citric acid	0.27	NS	0.29	<0.05
Decreased				
Leucine	-0.31	<0.04	-0.31	<0.03
UM13a	-0.18	NS	-0.29	<0.04
Glycine	-0.07	NS	-0.34	<0.02
Concentration regression model: R^2=0.520, F=3.255, $P<0.003$ Variables: 1) 3-methylhistidine $P<0.04$, 2) leucine -$P<0.008$, 3) UM13a -$P<0.07$				
Relative abundance regression model: R^2=0.527, F=3.347, $P<0.003$ Variables: 1) glycine -$P<0.006$, 2) UM13a -$P<0.04$, 3) 3-methylhistidine $P<0.03$				
Depression Dimension				
All study subjects	r	*P*	r	*P*
Increased				
Citric acid	0.27	NS	0.30	<0.04
Decreased				
Glycine	-0.09	NS	-0.33	<0.02
Concentration regression model: R^2=0.702, F=3.926, $P<0.0005$ Variables: 1) 3-methylhistidine $P<0.06$, 2) threonine -$P<0.03$, 3) citric acid $P<0.08$				
Relative abundance regression model: R^2=0.523, F=2.666, $P<0.01$ Variables: 1) glycine -$P<0.01$, 2) 3-methylhistidine -$P<0.21$, 3) citric acid $P<0.11$				

3.4.1.1. Differences in Urinary Metabolites in Relationship to SCL-90-R Depression.

Axis II of the RDC/TMD criteria requires the assessment of the SCL-90-R depression dimension. Within the RDC/TMD type 1a patients the SCL-90-R depression dimension was correlated with symptom prevalence (r=0.70, $P<0.003$), pain severity (r=0.70, $P<0.003$) and body pain distribution (r=0.67, $P<0.004$), but not duration of the illness (r=0.38). Thus the depression dimension score was positively associated with increasing severity of symptoms. Multiple regression analysis was applied to determine the associations between the SCL-90-R depression dimension and the urine excretion profiles and is shown in Table 3.9. Table 3.9 also shows that only glycine and citric acid were correlated when all study subjects were assessed. No correlation was found between depression and any metabolite within the RDC/TMD type 1a group. This shows that the biochemical changes associated with depression dimension scores are different from those associated with TMD symptom expression, which is consistent with the symptom-associated findings presented in Chapter 2. These data do not support a strong relationship between depression as assessed by the methods required in the RDC/TMD Axis II criteria and TMD.

3.4.2. TMD Pain in CFS Study.

3.4.2.a. Patient Characteristics.

A total of 100 CFS patients were recruited and 83 age- and sex-matched control subjects were recruited as presented in Chapter 2. There was no difference in any characteristics when comparing the 100 CFS patients (age = 29.1±12.5 years; range 13-73 years; % female 73.0) with the 83 control subjects (age = 32.8±13.9 years; range 14-69 years; % female 72.3).

3.4.2.b. Prevalence of TMD Symptoms.

As detailed in chapter 2, 29 (29%) of the CFS patients reported facial pain to the clinicians, whilst 19% of the controls compared with 69% of CFS patients reported TMD symptoms in their questionnaire ($P<0.0001$). Eleven percent of control subjects and 23% of CFS patients reported either facial or TMJ pain ($P<0.04$) and 8% of control subjects and 46% of CFS patients reported both facial muscle and TM joint pain ($P<0.0001$). The CFS patients with facial pain (FP) had an increased prevalence of infectious symptoms: fever (FP=55%; NoFP=32% - $P<0.02$), sore throat (FP=77%; NoFP=57% - $P<0.04$), and cervical lymphodynia (FP=66%; NoFP=45% - $P<0.04$), as previously reported in Chapter 2.

3.4.2.c. Gross Urine Parameters.

The overnight collection of urine was assessed for differences in volume and metabolite excretion. The time period between the last urination in the evening and the sample collection time in the morning was similar in both control and CFS groups (CFS=426±12.6 minutes; C=427±13.8). The CFS group had a reduction in the urine volume excreted per minute (CFS=0.64±0.03 mL/min; C=0.76±0.04 mL/min; $P<0.009$).

In the CFS group there was a positive correlation between urine volume excreted per minute and, the total metabolite ($r=0.39$, $P<0.001$) and total amino acids excreted per minute ($r=0.29$, $P<0.004$). No correlation was found between urine volume and the total organic acids excreted per minute ($r=0.10$).

3.4.2.d. Urine Amino and Organic Acids.

Table 3.10 shows a summary of the multiple regression and univariate analyses of the differences in urinary excretion rate per minute and relative abundance metabolites between the CFS patients and the control subjects. The primary regression discriminating metabolite was a reduction in asparagine followed by an increase in acetaminophen (Panadol). Table 3.10 also shows the t-test analysis of the urine metabolite data with elevations in the excretion rates and relative abundance of the proteolysis markers, tyrosine, 3-methylhistidine and the tyrosine: leucine ratio. The excretion rates of proteolysis control amino acids including leucine, alanine, asparagine, phenylalanine, valine and proline, were reduced. Furthermore reductions in succinic acid, UM17, hippuric acid and s-methylcysteine, and increases in UM27, UM28 and 1-methylhistidine were also noted. Importantly, with the exception of tyrosine, those amino or organic acids associated with the argininosuccinate/aspartate shunt (asparagine, succinic acid, and phenylalanine) and glycolysis input into the citric acid cycle (alanine), are all reduced.

The tyrosine: leucine ratio was 2.32±5.15 in the control group and 3.09±4.28 in the CFS group. Twenty-nine control subjects did not report any muscle pain over the previous 12-months and this group had a mean tyrosine: leucine ratio of 1.26 (Standard Error = 0.34, Standard Deviation =1.82) that was very similar to the pain free RDC/TMD type 1a control group (Mean = 1.13, Standard Error = 0.23, Standard Deviation =1.21). Forty-eight CFS patients had a muscle pain score >2 and their mean tyrosine: leucine ratio was 4.00 (Standard Error = 0.78, Standard Deviation =5.39) which was less than the mean tyrosine: leucine ratio of the RDC/TMD type 1a group (Mean = 7.21, Standard Error = 1.91, Standard Deviation

=6.62). These data show that in patients with muscle pain (both the RDC/TMD and CFS study groups) that the 2 Standard Deviation range for the tyrosine: leucine ratio was between 3.55 and 4.9, a mean value of 4.2.

Table 3.10. Summary of the multiple regression and t-test analysis of the changes in urine metabolites in CFS patients compared with the control group.

Variable	Excretion rate/minute			Relative Abundance		
	Control (n=81) X (SE)	CFS (n=96) X (SE)	*P*	Control (n=82) X(SE)	CFS (n=99) X (SE)	*P*
Tyrosine: leucine	2.32(5.15)	3.09(4.28)	<0.006			
Increased						
Acetaminophen	2.5 (1.1)	77.0 (32.3)	<0.004	0.09 (0.04)	1.93 (0.67)	<0.004
3-Methylhistidine	84.8 (11.5)	107.7 (13.9)	<0.03	1.81 (0.19)	2.56 (0.19)	<0.002
Tyrosine	24.9 (3.8)	30.4 (3.8)	<0.03	0.50 (0.06)	0.79 (0.08)	<0.003
UM27	67.3 (12.7)	85.1 (13.3)	<0.05	1.20 (0.23)	1.90 (0.23)	<0.01
1-Methylhistidine	91.1 (24.2)	145.2 (32.1)	NS	1.83 (0.47)	3.50 (0.67)	<0.03
UM28	4.6 (0.8)	6.2 (1.0)	NS	0.08 (0.01)	0.15 (0.02)	<0.05
Decreased						
Asparagine	57.6 (4.8)	35.7 (3.5)	<0.00003	1.76 (0.16)	1.17 (0.11)	<0.002
Succinic acid	34.3 (2.9)	23.7 (2.5)	<0.0001	0.97 (0.09)	0.69 (0.05)	<0.003
Phenylalanine	36.5 (2.8)	28.0 (2.6)	<0.003	0.94 (0.05)	0.80 (0.05)	<0.02
UM17	5.0 (0.5)	3.4 (0.4)	<0.004	0.13 (0.01)	0.10 (0.01)	<0.03
Hippuric acid	1009.1 (124.0)	807.2 (130.4)	<0.02	20.75 (1.56)	16.54 (1.53)	<0.03
Proline	74.1 (6.9)	56.8 (3.4)	<0.02	2.03 (0.14)	1.77 (0.11)	NS
Alanine	236.2 (16.8)	193.1 (14.8)	<0.02	6.30 (0.39)	5.60 (0.31)	NS
Valine	37.0 (2.4)	31.0 (2.0)	<0.02	1.03 (0.06)	0.92 (0.05)	NS
Leucine	14.7 (1.0)	12.1 (0.8)	<0.03	0.40 (0.02)	0.36 (0.02)	NS
S-Methylcysteine	10.7 (1.2)	7.8 (0.9)	<0.02	0.26 (0.02)	0.23 (0.02)	NS
Excretion rate/minute regression model: R^2=0.318, F=4.724, *P*<0.00000						
Variables: 1) asparagine -*P*<0.00007, 2) acetaminophen *P*<0.04, 3) 3-methylhistidine *P*<0.06.						
Relative abundance regression model: R^2=0.279, F=3.970, *P*<0.00000						
Variables: 1) asparagine -*P*<0.002, 2) hippuric acid -*P*<0.009, 3) acetaminophen -*P*<0.04.						

3.4.2.e. Urine Amino and Organic acids and Gross Urine Data.

Table 3.11 shows the summary of the multiple regression and correlation analyses between urinary volume and the excretion rate per minute of amino and organic acids within the CFS patients. Glutamic acid was the primary regression variable for urine volume. Different multiple regression parameters were associated with the different gross urine parameter assessments (total metabolite, amino acid and organic acids excreted per minute - Table 3.11). The urinary tyrosine: leucine ratio was positively correlated with all the gross urine assessments although most strongly correlated with urine volume. Table 3.12 shows the summary of multiple regression and correlation statistics for the urine relative abundance of amino and organic acids with the gross urine parameters. Table 3.12 shows that only 3 metabolites were altered in relative abundance with urine volume. An increase in the relative abundance of lysine was the principle alteration in relationship to the change in urine volume in the CFS patients.

Table 3.11. Summary of the multiple regression and correlation analyses of the association between the urinary volume, metabolite, amino acid and organic acid excretion parameters.

Serum Parameter	**mL/Minute**	**Metabolite/ Minute**	**Organic acids/ Minute**	**Amino acids/ Minute**
Urine	r P	r P	r P	r P
Tyrosine: leucine ratio	0.28 <0.007	0.25 <0.02	0.23 <0.03	0.26 <0.02
Excretion rate/minute				
Glutamic acid	0.37 <0.001	0.76 <0.001	0.55 <0.001	0.82 <0.001
Threonine	0.34 <0.001	0.69 <0.001	0.32 <0.002	0.87 <0.001
Proline	0.34 <0.001	0.57 <0.001	0.28 <0.006	0.76 <0.001
Lysine	0.34 <0.001	0.65 <0.001	0.47 <0.001	0.67 <0.001
Aspartic acid	0.33 <0.001	0.77 <0.001	0.55 <0.001	0.80 <0.001
β-alanine	0.32 <0.001	0.50 <0.001	0.26 <0.02	0.65 <0.001
Valine	0.32 <0.002	0.56 <0.001	0.23 <0.03	0.79 <0.001
UM15a	0.30 <0.003	0.58 <0.001	0.51 <0.001	0.60 <0.001
Phenylalanine	0.29 <0.005	0.65 <0.001	0.43 <0.001	0.70 <0.001
Hydroxyproline	0.28 <0.005	0.62 <0.001	0.42 <0.001	0.70 <0.001
Glycine	0.28 <0.005	0.70 <0.001	0.39 <0.001	0.87 <0.001
Alanine	0.27 <0.007	0.58 <0.001	0.25 <0.02	0.80 <0.001
Serine	0.26 <0.02	0.63 <0.001	0.32 <0.002	0.84 <0.001
Ornithine	0.25 <0.02	0.64 <0.001	0.50 <0.001	0.67 <0.001
Leucine	0.23 <0.03	0.57 <0.001	0.31 <0.002	0.72 <0.001
Asparagine	0.23 <0.03	0.28 <0.006	-	0.35 <0.001
β-aminoisobutyrate	0.22 <0.04	0.36 <0.001	-	0.51 <0.001
3-Methylhistidine	-	0.65 <0.001	0.62 <0.001	0.57 <0.001
Aconitic acid	-	0.64 <0.001	0.63 <0.001	0.48 <0.001
Citric acid	-	0.59 <0.001	0.71 <0.001	0.29 <0.004
Ethanolamine	-	0.54 <0.001	0.45 <0.001	0.59 <0.001
Succinic acid	-	0.50 <0.001	0.41 <0.001	0.46 <0.001
Tyrosine	-	0.49 <0.001	0.46 <0.001	0.42 <0.001
UM13a	-	0.48 <0.001	0.42 <0.001	0.43 <0.001
UM14	-	0.45 <0.001	0.27 <0.009	0.56 <0.001
UM27	-	0.44 <0.001	0.57 <0.001	0.22 <0.03
CFSUM1	-	0.42 <0.001	0.51 <0.001	0.20 <0.05
UM28	-	0.42 <0.001	0.51 <0.001	0.20 <0.05
UM15	-	0.41 <0.001	0.30 <0.003	0.46 <0.001
S-Methylcysteine	-	0.41 <0.001	0.32 <0.001	0.43 <0.001
UM13	-	0.41 <0.001	0.23 <0.03	0.53 <0.001
UM17	-	0.40 <0.001	0.38 <0.001	0.36 <0.001
Phenylacetic acid	-	0.34 <0.001	0.32 <0.001	0.26 <0.01
Hippuric acid	-	0.56 <0.001	0.79 <0.001	-
1-Methylhistidine	-	-	0.34 <0.001	-

mL/minute regression model; $R^2=0.376$, F=6.623, $P<0.00000$
Variables; 1) Glutamic acid $P<0.0007$, 2) Citric acid -$P<0.002$, 3) lysine $P<0.00005$
Metabolite/minute regression model; $R^2=0.922$, F=54.998, $P<0.00000$
Variables; 1) Aspartic acid $P<0.006$, 2) Hippuric acid $P<3.0E^{-11}$, 3) Glycine $P<0.00002$
Amino acids/minute regression model; $R^2=0.976$, F=145.76, $P<0.00000$
Variables; 1) Threonine $P<0.005$, 2) Glycine $P<1.0E^{-14}$, 3) Lysine $P<0.009$
Organic acids/minute regression model; $R^2=0.914$, F=43.156, $P<0.00000$
Variables; 1) Hippuric acid $P<1.0E^{-14}$, 2) Aspartic acid $P<0.03$, 3) Citric acid $P<0.11$

Thus, whilst there is a positive correlation between urine volume and the urine excretion rate per minute of 17 different metabolites (Table 3.11), only 3 metabolites changed in their relative abundance (Table 3.12). This was very similar to the metabolite correlations found between pain severity (VAS) and the concentration analysis in the RDC/TMD type 1a group (Table 3.2). This indicates that increases in facial pain severity are associated with an increase in urine volume and metabolite excretion without any change in urine concentration.

Table 3.12. Summary of the multiple regression and correlation analyses of the association between the urinary relative abundance of amino and organic acids and the urinary excretion parameters.

Serum Parameter	**mL/Minute**	**Metabolite/ Minute**	**Organic acids/ Minute**	**Amino acids/ Minute**
Relative Abundance	r *P*	r *P*	r *P*	r *P*
Increased				
Lysine	0.35 <0.001	0.38 <0.001	-	0.52 <0.001
Citric acid	-	0.33 <0.001	0.54 <0.001	-
Hippuric acid	-	0.22 <0.04	0.55 <0.001	-
UM28	-	-	0.43 <0.001	-
UM27	-	-	0.35 <0.001	-
1-Methylhistidine	-	-	0.22 <0.04	-
Variable				
Glycine	-	-	-0.43 <0.001	0.27 <0.007
Decreased				
Asparagine	-	-0.43 <0.001	-0.49 <0.001	-0.23 <0.03
UM17	-0.24 <0.02	-0.20 <0.05	-	-
CFSUM1	-0.25 <0.02	-	-	-0.34 <0.001
Proline	-	-0.55 <0.001	-0.69 <0.001	-
Valine	-	-0.54 <0.001	-0.73 <0.001	-
Leucine	-	-0.53 <0.001	-0.68 <0.001	-
Phenylalanine	-	-0.45 <0.001	-0.53 <0.001	-
Glutamic acid	-	-0.44 <0.001	-0.49 <0.001	-
Alanine	-	-0.36 <0.001	-0.61 <0.001	-
Serine	-	-0.34 <0.001	-0.56 <0.001	-
Aspartic acid	-	-0.33 <0.001	-0.44 <0.001	-
Succinic acid	-	-0.31 <0.002	-0.31 <0.003	-
β-aminoisobutyrate	-	-0.30 <0.004	-0.35 <0.001	-
Threonine	-	-0.29 <0.004	-0.61 <0.001	-
Ethanolamine	-	-0.27 <0.007	-0.23 <0.03	-
β-alanine	-	-0.25 <0.02	-0.36 <0.001	-
Hydroxyproline	-	-0.24 <0.02	-0.31 <0.002	-
Phenylacetic acid	-	-0.27 <0.009		-0.24 <0.02
UM14	-	-	-0.26 <0.02	-
S-Methylcysteine	-	-	-0.20 <0.05	-

ml/minute regression model; R^2=0.465, F=4.347, *P*<0.00000
Variables; 1) Lysine *P*<0.005, 2) UM27 *P*<0.87, 3) Serine -*P*<0.0003
Metabolite/minute regression model; R^2=0.695, F=10.600, *P*<0.00000
Variables; 1) Proline -*P*<0.04, 2) Lysine *P*<4.6E^{-8}, 3) Glutamic acid *P*<0.81
Amino acids/minute regression model; R^2=0.713, F=10.072, *P*<0.00000
Variables; 1) Lysine *P*<1.4E^{-13}, 2) Glycine *P*<0.000002, 3) Proline -*P*<0.008
Organic acids/minute regression model; R^2=0.808, F=17.092, *P*<0.00000
Variables; 1) Proline -*P*<0.02, 2) Hippuric acid *P*<3.0E^{-8}, 3) Citric acid *P*<1.5E^{-7}

3.4.2.f. Serum Amino and Organic Acids.

Table 3.13 summaries the multiple regression and t-test analyses of the differences in serum concentration and relative abundance of the amino and organic acids between the CFS patients and the control subjects. Multiple regression revealed that the primary discriminating metabolite was the increased level of unknown Ph2a and decrease in β-aminoisobutyrate levels. Table 3.13 also shows the t-test analysis of the serum metabolite data with elevations in the concentration and relative abundance of unknown Ph2a and ethanolamine along with reductions in phenylalanine, tyrosine and the two β-amino acids, β-aminoisobutyrate and β-alanine. The serum tyrosine: leucine ratio was reduced in the CFS group.

3.4.2.g. Serum Biochemistry, and Red and White Blood Cell Changes.

Table 3.14 summarises the differences in standard red and white blood cell profiles between CFS patients and control subjects. Discriminant function analysis revealed that the blood cell profiles were different between CFS patients and control subjects. The standard discriminant function analysis indicated that the primary factors differentiating the CFS patients from controls included the neutrophil: lymphocyte ratio, the % neutrophils, the % lymphocytes and the lymphocyte count. Both the neutrophil count and the neutrophil: lymphocyte ratio were elevated in the CFS patients compared with the controls. No CFS or control subject had any blood cell parameter that was outside the normal laboratory 2 standard deviation range. Two (2.4%) of the control subjects had an elevated neutrophil: lymphocyte ratio compared with 15 (15.2%) of the CFS patients ($P<0.004$), whilst no subject in either group had a neutrophil: lymphocyte ratio below the 2 standard deviation range (0.70 – 2.66) for the control group. Thus the principle change noted by the regression analysis was an increase in the neutrophil: lymphocyte ratio which occurred to an overt degree in 15% of the CFS patients.

Standard serum biochemistry was obtained from the CFS patients but not the control subjects and therefore could not be assessed (Table 3.14). However none of the variables were considered to be outside the normal 2 standard deviation range nor indicative of pathology.

Table 3.13. Multiple regression and t-test analysis of the changes in serum metabolites between CFS patients and control subjects.

Metabolite	**Excretion rate/ minute**			**Relative Abundance**		
Variable	Control X (SE)	CFS X (SE)	*P*	Control X (SE)	CFS X (SE)	*P*
Tyrosine: leucine	0.43	0.34	<0.00009			
Increased						
Ph2a	7023(749)	12231(743)	$<2.0E^{-8}$	0.54(0.06)	1.06(0.06)	$<1.0E^{-9}$
Ethanolamine	6985(474)	8421(451)	<0.005	0.54(0.04)	0.72(0.04)	<0.03
Ph1	31543(1507)	33959(2181)	<0.05	2.35(0.11)	2.81(0.18)	NS
Decreased						
Phenylalanine	64735(3008)	53245(3167)	<0.00002	4.56(0.15)	3.79(0.10)	<0.000006
Tyrosine	35812(1563)	27192(1785)	<0.00004	2.54(0.09)	1.96(0.10)	<0.000006
β-Aminoisobutyrate	12532(504)	10282(494)	<0.009	0.91(0.03)	0.80(0.03)	<0.007
β-Alanine	1656(198)	1149(94)	<0.02	0.11(0.01)	0.08(0.01)	<0.05
No Change						
Serine	40321(2212)	40289(2409)	NS	2.92(0.13)	2.95(0.10)	NS
Alanine	174389(5271)	169575(6260)	NS	12.84(0.29)	13.55(0.28)	NS
Glycine	90763(3057)	89494(4069)	NS	6.64(0.16)	6.90(0.18)	NS
Valine	135092(3778)	121282(4265)	NS	9.88(0.17)	9.77(0.21)	NS
Threonine	82377(3410)	73285(2986)	NS	5.82(0.21)	5.75(0.16)	NS
Aminohydroxyproprionate	19328(1556)	18344(1540)	NS	1.45(0.12)	1.49(0.11)	NS
Ph2	13209(1008)	10874(688)	NS	0.98(0.07)	0.94(0.06)	NS
Leucine	83787(2551)	73923(2876)	NS	6.10(0.12)	5.85(0.11)	NS
Isoleucine	40628(1276)	38172(1475)	NS	2.96(0.06)	3.00(0.06)	NS
Proline	111755(6070)	123649(7605)	NS	8.02(0.36)	8.81(0.37)	NS
S-methylcysteine	716(206)	1188(262)	NS	0.05(0.01)	0.08(0.01)	NS
Asparagine	4407(407)	3734(345)	NS	0.32(0.03)	0.26(0.02)	NS
Aspartic acid	45885(2583)	42056(3143)	NS	3.25(0.13)	2.92(0.13)	NS
Ornithine	66173(3872)	58208(4004)	NS	4.73(0.28)	4.44(0.27)	NS
Glutamic acid	252041(10451)	241022(13850)	NS	17.87(0.44)	17.37(0.44)	NS
Lysine	55308(3360)	56132(3789)	NS	3.85(0.18)	4.04(0.17)	NS
Aconitic acid	4798(388)	5743(548)	NS	0.34(0.02)	0.40(0.03)	NS
Tryptophan	4215(450)	3290(390)	NS	0.28(0.03)	0.22(0.02)	NS
Concentration regression model: R^2=0.272, F=10.773, P<0.00000; Variables: 1) Ph2a P<0.00001, 2) β-aminoisobutyrate -P<0.0005, 3) ornithine P<0.06.						
Relative abundance regression model: R^2=0.312, F=7.650, P<0.00000; Variables: 1) Ph2a P<0.002, 2) β-aminoisobutyrate -P<0.003 3) tyrosine -P<0.09						

Table 3.14. Red and White Blood Cell Parameters and Pesticide levels for CFS Patients Compared with Control Subjects using Students t-test and Standard Discriminant Function Analyses.

Variable	CFS X (S.D.)	Control X (S.D.)	P (t-test)	Prevalence CFS Abnormal
Red cell count (10^{12}/L)	4.61 (0.40)	4.62 (0.41)	NS	NS
Haemoglobin (g/L)	13.85 (1.10)	13.69 (1.15)	NS	NS
Haematocrit (L/L)	40.44 (3.19)	40.07 (3.37)	NS	NS
Mean corpuscular volume (fL)	87.74 (3.16)	86.75 (3.77)	NS	NS
Mean cell haemoglobin (pg)	30.07 (1.30)	29.68 (1.49)	NS	NS
Mean cell haemoglobin conc. (g/100mL)	34.25 (0.62)	34.17 (0.59)	NS	NS
Red cell distribution width (%)	12.35 (0.94)	12.58 (0.83)	NS	NS
Platelet count (10^9/L)	253.1 (60.7)	256.1 (62.9)	NS	NS
Mean platelet volume (fL)	8.60 (0.99)	8.36 (0.98)	NS	NS
Leukocyte count (10^9/L)	6.11 (1.44)	5.74 (1.36)	NS	NS
Lymphocyte count (10^9/L)	1.95 (0.56)	1.93 (0.60)	NS	NS
Neutrophil count (10^9/L)	3.46 (1.10)	3.12 (0.84)	<0.03	15 <0.004
Neutrophil : Lymphocyte ratio	1.91 (0.80)	1.68 (0.49)	<0.03	NS
Monocyte count (10^9/L)	0.48 (0.15)	0.47 (0.14)	NS	NS
Eosinophil count (10^9/L)	0.19 (0.15)	0.20 (0.18)	NS	NS
Basophil count (10^9/L)	0.02 (0.02)	0.02 (0.03)	NS	NS
% Neutrophils	56.17 (8.66)	54.07 (6.87)	NS	NS
% Lymphocytes	32.60 (8.02)	33.78 (6.22)	NS	NS
% Monocytes	7.86 (1.55)	8.36 (1.80)	NS	NS
% Eosinophils	3.08 (2.09)	3.36 (2.69)	NS	NS
% Basophils	0.33 (0.28)	0.38 (0.47)	NS	NS
Pesticides	X(prev %)	X(prev %)	Mean	Prevalence
DDE	3.73 (96.9%)	2.98 (97.5%)	NS	NS
HCB	0.24 (12.4%)	0.48 (27.5%)	<0.03	<0.02
Heptochlor	0.19 (11.3%)	0.05 (5.0%)	NS	NS
Deidrin	0.17 (9.3%)	0.06 (5.0%)	NS	NS
β-BHC	0.04 (5.2%)	0.11 (10.0%)	NS	NS
Aldrin	0.04 (3.1%)	0.01 (1.3%)	NS	NS
α-BHC	0.01 (1.0%)	0	NS	NS
DDT	0	0	NS	NS
Discriminant Function Analyses				
Blood cell parameters: Wilks' λ = 0.801, F=1.868, P<0.02				
Pesticides: Wilks' λ = 0.943, F=2.592, P<0.04				

3.4.2.h. Pesticides.

Table 3.14 shows that the CFS patients did not have any alteration in pesticide levels apart from a reduction in the levels and prevalence of detectable HCB. The discriminant function analysis showed a difference with HCB being the primary discriminant parameter.

3.4.2.i. Reported facial pain.

The 29 CFS patients who reported facial pain were compared with the remaining CFS patients to assess which blood cell and biochemical parameters were associated with the reporting of pain. Table 3.15 shows that forward stepwise multiple regression found a difference in the parameter profiles between the CFS facial pain group and the remaining CFS patients. The primary parameter that determined the difference was the ANA titre. Univariate analysis showed that the CFS facial pain patients had an increased prevalence of a positive ANA response (P<0.0005) and elevated ANA titre (P<0.0001) compared with the remaining CFS patients. These CFS facial pain patients also had increases in mean red cell volume, serum unknown Ph1, urinary relative abundance of lysine and the excitatory amino acid, aspartic acid, and a reduction in serum proline.

Table 3.15. Summary of the multiple regression and univariate analyses of the association between the biochemical parameters and the reporting of facial pain in CFS patients.

Parameter	Facial Pain	No Facial Pain	P
Increased	% or X(SD)	% or X(SD)	
ANA	68%	25%	<0.0005
ANA Titre	115.2(205)	17.8(42)	<0.0001
Mean corpuscular Volume (MCV)	89.1(2.9)	87.4(3.1)	<0.02
Serum Ph1	37923(25091)	32758(20713)	<0.02
% Urine Lysine	7.13(14.8)	2.73(2.71)	<0.04
% Urine Aspartic acid	1.76(0.92)	1.39(0.58)	<0.05
Decreased			
Proline	95228(70848)	133090(76263)	<0.009
Facial pain regression model; R^2=0.400, F=2.670, P<0.02			
Variables; 1) ANA titre P<0.005, 2) Serum Ph1 P<0.09, 3) ERPM Urinary Hippuric acid P<0.14.			

3.4.2.j. 7-Day Facial Pain Severity.

Table 3.16 shows the univariate analysis of those CFS patients who gave a positive response to the 7-day severity of facial pain question compared with the control subjects who did not respond positive to either of the 7-day facial pain or TMJ pain severity questions. The CFS facial pain group had an increase in the percentage neutrophils and the urinary tyrosine: leucine ratio as well as a reduction in the serum tyrosine: leucine ratio. The serum levels of Ph2a and ethanolamine were increased whilst the phenylalanine level was reduced. They also had increases in the excretion rate per minute and relative abundance of acetaminophen and tyrosine. There was a reduction in the excretion rate per minute of asparagine and the relative abundance of UM17 and an increase in the relative abundance of UM15a.

Within the CFS group and the control group the 7-day facial pain scalar response was used to assess which blood cell and biochemical parameters were associated with the reporting of facial pain. Table 3.16 shows that forward stepwise multiple regression (all blood cell and biochemistry variables included) found a difference in the parameter profiles between the CFS facial pain group and the remaining CFS patients. The primary parameter that determined the difference in 7-day facial pain severity was a fall in the serum sodium concentration, even though it was in the normal laboratory 2 standard deviation range. Univariate analysis showed that the 7-day facial pain severity response was associated with reductions in serum sodium, chloride and 3-methylhistidine.

Table 3.16. Summary of the multiple regression and univariate analyses of the association between the biochemical parameters and the 7-day severity of facial pain in CFS patients and control subjects.

Serum Parameter	**Facial Pain**	**No Facial Pain**
T-test 7-day Facial pain positive v controls	X(SE)	X(SE)
Increased		
Serum Ph2a	10218(7344)	4433(3953) <0.007
Serum Ethanolamine	8451(3962)	5968(2708) <0.01
% Neutrophils	58.4(8.0)	52.4(8.3) <0.03
Urinary tyrosine: leucine ratio	2.32(1.23)	1.51(2.5) <0.03
% and ERPM Acetaminophen	0.91(2.38)	0 <0.03
% and ERPM Tyrosine	0.78(0.65)	0.41(0.58) <0.03
Decreased		
Serum Phenylalanine	57231(30147)	65218(20369) <0.05
Serum tyrosine: leucine ratio	0.37(0.16)	0.48(0.16) <0.05
% UM17	0.08(0.05)	0.15(0.10) <0.009
% UM15a	0.91(0.80)	0.44(0.30) <0.03
ERPM Asparagine	53.6(86.4)	59.6(38.1) <0.05
Correlation analysis	**CFS**	**Control**
Increased	r *P*	r *P*
% Urine Glutamine/Glutamic acid	-0.09 NS	0.35 <0.02
% Urine Serine	-0.01 NS	0.28 <0.05
Urine volume	0.03 NS	0.28 <0.05
Decreased		
Sodium	-0.29 <0.02	-
Chloride	-0.25 <0.05	-
Haemoglobin	-0.25 <0.05	-
% Urine 3-Methylhistidine	-0.25 <0.05	0.11 NS
7-Day facial pain severity in CFS patients regression model; R^2=0.483, F=2.708, P<0.02 Variables; 1) Sodium -P<0.005, 2) Serum glycine P<0.14, 3) Serum tyrosine P<0.008.		
7-Day facial pain severity in Control subjects regression model; R^2=0.941, F=16.665, P<0.00000 Variables; 1) % U Glutamic acid P<0.0004, 2) Serum β-aminoisobutyrate -P<0.00003, 3) DDE P<0.00006.		

Multiple regression and univariate analyses (excluding the serum chemistry) were also applied to the 7-day facial pain severity response in the control subjects. The primary regression variable in the control subjects for 7-day facial pain was the % glutamic acid, followed by a reduction in serum β-aminoisobutyrate and an increase in the pesticide DDE. The 7-day response was associated with an increase in urine volume and increases in the relative abundance of the excitatory amino acid, glutamic acid and the phospholipid head-group amino acid, serine. These changes are very similar to those for pain severity in the RDC/TMD type 1a group.

3.4.2.k. 12-Month Facial Pain Severity.

Table 3.17 shows the forward stepwise multiple regression analysis of the blood cell and biochemistry variables in relationship to the scalar response to 12-month facial pain severity within the CFS patients.

Table 3.17. Summary of the multiple regression and univariate analyses of the association between the biochemical parameters and the 12-month severity of facial pain in CFS patients and control subjects.

Correlation Analysis	CFS	Control
Serum Increased	r P	r P
ALT	0.40 <0.001	-
AST	0.36 <0.001	-
Basophil count	0.29 <0.008	-0.12 NS
% and ERPM Urinary Aspartic acid	0.28 <0.02	-0.05 NS
Erythrocyte Sedimentation rate (ESR)	0.27 <0.02	-
Protein	0.27 <0.02	-
Urine volume	0.27 <0.02	0.04 NS
Serum β-Alanine	0.24 <0.03	0.06 NS
% and ERPM Urinary Acetaminophen	0.24 <0.03	-0.09 NS
ERPM Urinary Glutamic acid	0.23 <0.04	-0.05 NS
Neutrophil count	0.23 <0.04	-0.11 NS
Leukocyte count	0.22 <0.05	-0.17 NS
Serum Glutamic acid	0.22 <0.04	0.19 NS
Pesticide residue DDE	0.22 <0.05	0.16 NS
Decreased		
% and ERPM Urinary Hippuric acid	-0.24 <0.04	0.12 NS
Serum Isoleucine	-0.23 <0.04	-0.08 NS
Serum Proline	-0.22 <0.05	-0.08 NS
% Urinary UM27	-0.22 <0.05	-0.11 NS
ERPM Urinary CFSUM1	-0.22 <0.05	0.09 NS
ERPM Urinary UM28	-0.22 <0.05	0.09 NS
Serum Ph1	0.07 NS	-0.29 <0.02
Serum β-Aminoisobutyrate	0.13 NS	-0.26 <0.03
12-month facial pain severity CFS patient regression model; R^2=0.648, F=6.456, P<0.00001		
Variables; 1) ALT P<0.28, 2) ESR P<0.007, 3) Serum iron P<0.009.		
12-month facial pain severity Control subject regression model; R^2=0.908, F=7.765, P<0.00000		
Variables; 1) Serum Ph1-P<0.003, 2) % Eosinophils -P<0.003, 3) % urinary UM14 P<0.000000004.		

The primary parameter that determined the difference was an increase in serum ALT levels followed by the erythrocyte sedimentation rate (ESR). Univariate analysis (Spearman rank correlation) showed that in the CFS patients the 12-month facial pain severity response was associated with increases in the neutrophil and basophil counts, the ESR, the tissue damage markers ALT, AST, protein, and two serum amino acids which have neuronal excitatory capacity. Increases in the ml of urine excreted per minute and the excretion of the excitatory amino acids, aspartic acid and glutamic acid. Reductions in serum isoleucine and proline as well as urinary excretion of hippuric acid were also noted. Multiple regression and univariate analyses were also applied to the 12-month facial pain severity response in the control subjects. The principle parameter associated with the 12-month severity of facial pain in the control subjects was the reduction in the serum concentration of Ph1. The 12-month response in the control subjects was associated with decreases in serum unknown Ph1 and β-aminoisobutyrate.

3.4.2.I. 12-Month Facial Pain Frequency.

The CFS patients who responded positively to the 12-month facial pain frequency scalar response were compared with the remaining CFS patients to assess which blood cell and biochemical parameters were associated with the frequency of reporting of facial pain and are shown in Table 3.18. The primary parameter that determined the difference was an increase in the ESR followed by the excretion rate per minute of the excitatory amino acid, aspartic acid. Univariate analysis (Spearman rank correlation) showed that the 12-month frequency of facial pain was associated with increases in the ESR, the neutrophil and basophil counts, ALT, potassium, serum s-methylcysteine, urine volume, DDE and Deildrin, as well as increases in urinary excitatory amino acids, aspartic and glutamic acids. Reductions in the % lymphocytes and serum proline were also noted. Multiple regression and univariate analyses were also applied to the 12-month facial pain frequency response in the control subjects. The principle parameter associated with the 12-month severity of facial pain in the control subjects was the reduction in the serum concentration of Ph1. The 12-month frequency response was associated with reductions in serum leukocyte and lymphocyte counts, unknown Ph1 and β-aminoisobutyrate, as well as reductions in urinary relative abundance of tyrosine. Interestingly, the pesticides DDE and Deildrin were increased in those CFS patients reporting more frequent facial pain.

The biochemistry of facial pain differed when assessing either: 1) the reporting of facial pain as a significant symptom; 2) the scalar responses to the 7-day severity; and 3) the 12-month frequency and severity. There was a high degree of similarity between the 12-month frequency and severity responses but little association between the 7-day severity responses and the other parameters assessed. These data confirm that the biochemistry associated with facial pain on the day it is measured is different from the biochemical changes associated with its frequency of occurrence. Thus the daily response needs to be assessed as a separate event from the frequency and 12-month severity responses. The 7 day response should indicate the actual pain process whilst the 12-month frequency and severity responses should indicate either results of, or factors that effect, the 7-day response. Whilst the 7-day responses are highly associated with changes in sodium, chloride and catecholamine precursor changes the 12-month frequency and severity are highly associated with tissue damage markers and immune cell alterations as well as pesticide increases.

Table 3.18. Summary of the multiple regression and univariate analyses of the association between the biochemical parameters and the 12-month frequency of facial pain in CFS patients and control subjects.

Serum Parameter	**Facial pain**	**No Facial Pain**
T-Test Facial pain positive v remaining patients		
Increased		
% and ERPM Urinary Acetaminophen	2.67%	0.26% <0.001
Pesticide residue DDE	4.68	2.78 <0.005
Urinary Tyrosine: Leucine ratio	3.63	2.22 <0.02
% and ERPM Urinary Tyrosine	0.83%	0.53% <0.03
% Neutrophils	57.3%	54.4% <0.03
Serum Ph2a	11297	9222 <0.03
Neutrophil count	3.56	3.17 <0.05
Pesticide residue Deildrin	0.25	0.08 <0.05
Decreased		
% Urinary Phenylalanine	0.75%	0.91% <0.03
% Urinary Proline	1.64%	2.02% <0.03
% Urinary Asparagine	1.22%	1.64% <0.04
% Lymphocytes	31.3%	33.8% <0.04
Serum Phenylalanine	51809	61139 <0.04
Serum Tyrosine: Leucine ratio	0.34	0.39 <0.04
% Urinary Hydroxyproline	0.38%	0.51% <0.05
% Urinary Alanine	5.28%	6.19% <0.05
Correlation Analysis	**CFS**	**Control**
Increased	r *P*	r *P*
Erythrocyte Sedimentation rate (ESR)	0.30 <0.02	-
ALT	0.27 <0.02	-
Potassium	0.26 <0.02	-
Deildrin	0.25 <0.02	-0.09 NS
Urine volume	0.24 <0.03	0.06 NS
Basophil count	0.24 <0.03	-0.10 NS
Neutrophil count	0.24 <0.03	-0.17 NS
Serum S-Methylcysteine	0.24 <0.03	-0.13 NS
% Urinary Acetaminophen	0.24 <0.03	0.13 NS
% and ERPM Urinary Aspartic acid	0.24 <0.03	-0.13 NS
ERPM Urinary Glutamic acid	0.22 <0.05	-0.10 NS
DDE	0.22 <0.05	0.14 NS
Decreased		
% Lymphocytes	-0.22 <0.04	-0.03 NS
Serum Proline	-0.22 <0.05	-0.08 NS
% Urinary Hippuric acid	-0.22 <0.04	0.17 NS
Lymphocyte count	-0.03 NS	-0.24 <0.05
Leukocyte count	0.19 NS	-0.25 <0.04
% Urinary Tyrosine	0.17 NS	-0.25 <0.04
Serum Ph1	0.06 NS	-0.27 <0.03
Serum β-Aminoisobutyrate	0.11 NS	-0.25 <0.03
12-month facial pain frequency CFS patient regression model; R^2=0.597, F=5.176, P<0.0002. Variables: 1) ESR P<0.009, 2) Excretion rate/min Aspartic acid P<0.0004, 3) Serum iron P<0.002.		
12-month facial pain frequency Control subject regression model; R^2=0.973, F=8.440, P<0.00008 Variables: 1) Serum Ph1 -P<0.003, 2) Leukocyte count -P<0.15, 3) Serum threonine P<0.0009.		

3.4.2.m. 7-Day TMJ Pain Severity.

Table 3.19 shows the univariate analysis of those CFS patients who gave a positive response to the 7-day severity of TMJ pain compared with the control subjects who did not respond positive to either of the 7-day facial pain or TMJ pain severity questions.

Table 3.19. Summary of the multiple regression and univariate analyses of the association between the biochemical parameters and the 7-day severity of TMJ pain in CFS patients and control subjects.

Serum Parameter	**TMJ Pain**	**No TMJ Pain**
T-test 7-day TMJ pain positive v controls		
Increased		
Serum Ph2a	12598(8542)	4433(3954) <0.0007
Serum Ethanolamine	9033(4479)	5968(2709) <0.008
% Urinary Tyrosine	0.99(1.03)	0.41(0.58) <0.01
% Neutrophils	58.1(5.6)	52.4(8.3) <0.02
Urinary tyrosine: leucine ratio	2.75(2.24)	1.51(2.50) <0.02
ERPM Urinary Tyrosine	42.0(36.5)	22.9(38.1) <0.02
ERPM Urinary Acetaminophen	26.7(75.9)	0.02(0.1) <0.04
% Urinary UM15a	0.94(0.96)	0.44(0.30) <0.04
Serum Ph1	35794(22203)	26597(11744) <0.05
Decreased		
% Urinary UM17	0.09(0.10)	0.15(0.10) <0.02
Serum Phenylalanine	57357(30552)	65218(20369) <0.03
Serum β-aminoisobutyrate	9606(4066)	12360(3720) <0.03
Serum tyrosine: leucine ratio	0.37(0.18)	0.48(0.16) <0.04
Correlation Analysis	**CFS**	**Control**
Increased	r *P*	r *P*
Serum Ph1	0.36 <0.02	0.09 NS
Serum Bilirubin	0.25 <0.05	-
% and ERPM Urinary Acetaminophen	-0.02 NS	0.55 <0.001
% Urinary β-aminoisobutyrate	-0.11 NS	0.37 <0.009
% Urinary UM15a	0.15 NS	0.32 <0.03
ERPM Urinary UM15	0.07 NS	0.28 <0.03
7-Day TMJ pain severity CFS patients regression model; R^2=0.612, F=4.575, P<0.0007 Variables; 1) Excretion rate/min UM15 P<0.22; 2) bilirubin P<0.0008; 3) sodium -P<0.0007 **7-DayTMJ pain severity Control subject** regression model: R^2=0.928, F=13.619, P<0.00000 Variables; 1) % urinary β-aminoisobutyrate P<0.00002; 2) serum proline P<0.000001; 3) Serum Ph2a P<0.00003		

The CFS TMJ pain group had an increase in the percentage neutrophils and the neutrophil: lymphocyte ratio as well as the urinary tyrosine: leucine ratio and a reduction in the serum tyrosine: leucine ratio. The serum levels of Ph1, Ph2a and ethanolamine were increased whilst the phenylalanine and β-aminoisobutyrate levels were reduced. They also had increases in the excretion rate per minute and relative abundance of tyrosine, as well as a reduction in the excretion rate per minute of acetaminophen and the relative abundance of UM17 and an increase in the relative abundance of UM15a.

The scalar 7-day TMJ pain response was assessed within the CFS patients and the control subjects. Table 3.19 indicates that forward stepwise multiple regression (all blood cell and biochemistry variables included) for the 7-day TMJ pain response was associated with differences in the other variables in both the CFS patients and the control subjects. In the CFS patients the primary parameter that determined the difference was an increase in the excretion rate per minute of UM15 followed by the bilirubin concentration. Univariate analysis (Spearman rank correlation) showed that the 7-day TMJ pain severity response was associated with increases in serum bilirubin and unknown Ph1. In the control subjects the primary variable for the forward stepwise regression analysis was the % urinary β-aminoisobutyrate. Univariate analysis was also applied to the 7-day TMJ pain severity response in the control subjects. The 7-day response was associated with an increase in UM15, the analgesic acetaminophen (paracetamol), β-aminoisobutyrate, UM15a, the percentage neutrophils and the neutrophil: lymphocyte ratio.

3.4.2.n. 12-Month TMJ Pain Severity.

The CFS patients who responded positively to the 12-month TMJ pain severity scalar response were compared with the remaining CFS patients to assess which blood cell and biochemical parameters were associated with TMJ pain. Similarly the scalar responses to the 12-month severity of TMJ pain were assessed by multivariate and univariate analyses in the CFS and control groups. Table 3.20 shows that the forward stepwise multiple regression analyses of the CFS patients (all blood cell and biochemistry variables included) and control subjects have different parameter profiles in association with the 12-month TMJ response. The primary parameter that determined the difference in the CFS patients was an increase in the erythrocyte sedimentation rate (ESR), followed by serum AST levels. Univariate analysis showed that in the CFS patients the 12-month TMJ pain severity response was associated with increases in the ESR, the tissue damage markers AST, protein, serum s-methylcysteine, the urine volume and the analgesic, acetaminophen. In the control group a fall in serum Ph1 and an increase in the relative abundance of aconitic acid were the primary parameters associated with the 12-month TMJ pain severity response in the control subjects.

3.4.2.o. 12-Month TMJ Pain Frequency.

The CFS patients who responded positively to the 12-month TMJ pain frequency scalar response were compared with the remaining CFS patients to assess which blood cell and biochemical parameters were associated with the frequency of reporting of TMJ pain.

Similarly the scalar responses to the 12-month frequency of TMJ pain were assessed by multivariate and univariate analyses in the CFS and control groups.

Table 3.20. Summary of the multiple regression and univariate analyses of the association between the biochemical parameters and the 12-month severity and frequency of TMJ pain in CFS patients and control subjects.

Serum Parameter	**CFS**	**Control**
Severity Correlation Analysis		
Increased	r *P*	r *P*
Erythrocyte Sedimentation rate (ESR)	0.30 <0.01	-
Urine volume	0.27 <0.02	0.04 NS
% and ERPM Urinary Acetaminophen	0.25 <0.03	-0.04 NS
Serum S-methylcysteine	0.24 <0.03	-0.09 NS
AST	0.23 <0.04	-
Protein	0.22 <0.04	-
% Urinary Aconitic acid	-0.07 NS	0.23 <0.05
Decreased		
Serum Ph1	0.17 NS	-0.23 <0.05
12-month TMJ pain severity CFS patient regression model; R^2=0.528, F=3.910, P<0.002 Variables; 1) ESR P<0.03, 2) AST P<0.003, 3) Serum β-aminoisobutyrate P<0.004. **12-month TMJ pain severity Control subject** regression model; R^2=0.985, F=23.020, P<0.00000 Variables; 1) serum Ph1 P<0.04, 2) % urine Aconitic acid P<0.04, 3) Serum aspartic acid P<0.03.		
Frequency **T-Test Analysis**	**TMJ Pain**	**No TMJ Pain**
Increased		
Serum Ph2a	11873	8375 <0.003
Serum S-Methylcysteine	1477	623 <0.006
MCV	88.2	86.7 <0.009
% and ERPM Urinary Acetaminophen	2.00%	0.29% <0.02
% and ERPM Urinary 3-Methylhistidine	2.47%	1.89% <0.02
% Urinary Ethanolamine	3.40%	2.58% <0.04
MCH	30.2	29.7 <0.04
Heptochlor	0.20	0.05 <0.04
Decreased		
Serum Tyrosine: Leucine ratio	0.33	0.41 <0.002
Serum Tyrosine	26424	33581 <0.008
Correlation Analysis	**CFS**	**Control**
Increased	r *P*	r *P*
Basophil count	0.27 <0.02	-0.07 NS
Mean corpuscular volume (MCV)	0.26 <0.02	0.05 NS
Erythrocyte Sedimentation rate (ESR)	0.25 <0.05	-
Eosinophil count	0.23 <0.04	-0.07 NS
Serum Glutamic acid	0.23 <0.04	0.06 NS
Serum S-methylcysteine	0.26 <0.02	-0.12 NS
Serum Lysine	0.26 <0.02	0.08 NS
Decreased		
Glycine	-0.29 <0.008	-0.11 NS
Ph1	0 NS	-0.25 <0.03
12-month TMJ pain frequency CFS patient regression model; R^2=0.535, F=4.031, P<0.001 Variables; 1) Serum glycine -P<0.25, 2) ESR P<0.01, 3) Serum s-methylcysteine P<0.02. **12-month TMJ pain frequency Control subject** regression model; R^2=0.999, F=195.11, P<0.00001 Variables; 1) Serum Ph1 P<0.00002, 2) Monocyte count -P<0.000009, 3) Serum tryptophan -P<0.00005.		

Table 3.20 shows that forward stepwise multiple regression analyses of the CFS patients (all blood cell and serum biochemistry and the urinary relative abundance variables included) and control subjects have different parameter profiles in association with the 12-month TMJ response. The primary parameter that determined the difference was a reduction in serum glycine, followed by the ESR. Univariate analysis (Spearman rank correlation) showed that the 12-month frequency of TMJ pain was associated with increases in the ESR, the basophil and eosinophil counts, mean red cell volume, serum s-methylcysteine, lysine and the excitatory amino acid, glutamic acid. A reduction in serum glycine levels was also noted. In the control subjects a reduction in serum unknown Ph1 was the principle factor associated with the increase in 12-month TMJ pain frequency.

3.4.2.p. Associations between Sodium, Symptoms and Biochemical Parameters.

A reduction in serum sodium was the primary factor associated with 7-day facial pain expression. To assess the associations between sodium, symptoms (Table 3.21) and biochemical changes (Table 3.22) the levels of sodium were compared using multi- and uni-variate analyses in the CFS patients

Table 3.21. Summary of the correlation analyses of the association between the SCL-90-R scalar symptom responses and serum sodium in the CFS patients.

SCL-90-R Question	7-Day Severity	12-month Frequency	12-month Severity
SODIUM	r *P*	r *P*	r *P*
Q40 Nausea or upset stomach	-0.31 <0.004	-0.29 <0.008	-0.23 <0.04
Q17 Trembling	-0.34 <0.001	-0.25 <0.03	-
Q111 Hair loss	-	-0.35 <0.001	-0.27 <0.02
Q36 Feeling others are unsympathetic or do not understand	-	-0.29 <0.008	-0.28 <0.01
Q114 Leg cramps	-	-0.24 <0.03	-0.22 <0.04
Q19 Poor appetite	-0.30 <0.005	-	-
Q115 Muscle twitches	-0.27 <0.03	-	-
Q123 Facial muscle pain or discomfort	-0.26 <0.05	-	-
Q52 Numbness or tingling in parts of your body	-0.24 <0.03	-	-
Q4 Faintness or dizziness	-0.22 <0.05	-	-
Q75 Feeling nervous when left alone	-0.21 <0.05	-	-
Q23 Suddenly scared for no reason	-	-0.22 <0.05	-
Q124 Swallowing difficulties	-	-0.22 <0.05	-
Q9 Trouble remembering things	-	-0.21 <0.05	-
Q103 Sore glands	-	-0.21 <0.05	-
Q42 Soreness of your muscles	-	-	-0.25 <0.03
Q113 Joint pain	-	-	-0.22 <0.04

7-day Severity regression model; R^2=0.953, F=26.436, P<0.00000
Variables; 1) Mind going blank -$P<1.0E^{-12}$, 2) Facial pain -$P<1.7E^{-10}$, 3) Heart racing/pounding P<0.000002
12-Month Frequency regression model; R^2=0.812, F=12.493, P<0.00000
Variables; 1) Hair loss -P<0.000001, 2) Swallowing difficulties -P<0.000004, 3) Q35 P<0.00002
12-Month Severity regression model; R^2=0.934, F=41.707, P<0.00000
Variables; 1) Hair loss -P<0.000001, 2) Others unsympathetic -P<0.000006, 3) Q24 P<0.05

The fall in serum sodium levels was associated with increases in the 7-day severity of dysregulated appetite, nausea, muscle symptoms (including facial pain), faintness and dizziness and paraesthesia. The fall in serum sodium levels was also associated with increases in the 12-month frequency and severity of nausea, musculoskeletal symptoms, hair loss and cognitive symptoms. These symptom associations with sodium are very similar to the symptoms associated with facial pain as shown in Tables 2.3 and 2.9 (Chapter 2; pages 26 and 36, respectively). These symptom data support the association between falling serum sodium levels and facial muscle pain.

Table 3.22 shows that the fall in serum sodium in the 7-day facial pain positive patients was associated with an increase in serum lysine levels and the platelet number, as well as reductions in creatinine and platelet volume.

Table 3.22. Summary of the differences in the correlation coefficients for sodium in CFS patients with a positive 7-day facial pain response compared with those with no response.

Parameter	Facial Pain		No Facial pain		Difference
	r	*P*	r	*P*	
Increased					
U % & EPM Succinic acid	0.75	<0.002	-0.17	NS	<0.002
U % & EPM β-alanine	0.71	<0.005	-0.30	<0.04	<0.0008
U % & EPM UM15	0.67	<0.009	-0.28	NS	<0.002
U % & EPM Glycine	0.66	<0.01	0.20	NS	NS
U EPM Leucine	0.65	<0.02	-0.26	NS	<0.003
U % & EPM Alanine	0.63	<0.02	-0.11	NS	<0.02
U EPM Valine	0.60	<0.03	-0.19	NS	<0.02
U EPM Phenylacetic acid	0.59	<0.03	-0.23	NS	<0.009
Creatinine	0.57	<0.04	0.16	NS	NS
Mean Platelet volume	0.55	<0.04	0.03	NS	NS
U EPM S-Methylcysteine	0.52	NS	-0.20	NS	<0.03
Amino acids / minute	0.50	NS	-0.16	NS	<0.04
Chloride	0.50	NS	0.29	<0.05	NS
ALT	0.48	NS	0.01	NS	NS
Iron	0.46	NS	-0.27	NS	<0.03
mls / minute	0.37	NS	-0.35	<0.02	<0.03
U % & EPM UM15a	0.35	NS	-0.36	<0.02	<0.04
Bicarbonate	0.09	NS	0.06	NS	NS
Decreased					
Organic acids / minute	0	NS	-0.29	<0.05	NS
U % & EPM Hippuric acid	-0.11	NS	-0.30	<0.04	NS
ESR	-0.55	NS	0.24	NS	<0.02
Platelet number	-0.56	<0.04	0.10	NS	<0.04
Serum Lysine	-0.61	<0.02	-0.04	NS	NS
U%, EPM 1-Methylhistidine	-0.36	NS	-0.27	NS	NS
Facial Pain regression model; R^2=0.973, F=14.163, *P*<0.07					
Variables; 1) % U glycine *P*<0.03, 2) Serum S-methylcysteine *P*<0.06, 3) % U UM15 *P*<0.27					
No Facial Pain regression model; R^2=0.941, F=6.433, *P*<0.02					
Variables; 1) urine volume -*P*<0.12, 2) iron *P*<0.05, 3) % U Hippuric acid *P*<0.002					

In the 7-day facial pain positive patients serum sodium was associated with reductions in either urinary excretion rate per minute and/or relative abundance of succinic acid, β-alanine, glycine, alanine, leucine, valine, phenylacetic acid and unknown UM15. In the CFS patients without a 7-day facial pain response falls in serum sodium were associated with increases in the urine volume and urine organic acids excreted per minute and increases in β-alanine, UM15a and hippuric acid and a reduction in serum chloride levels. Large correlation differences were found for 5 urinary metabolites, succinic acid, leucine, β-alanine, phenylacetic acid and UM15, whilst a total of 15 measures were different between the two groups. Between group dysregulation of urine volume and the amino acids excreted per minute was also seen.

Thus the fall in serum sodium associated with 7-day facial pain is associated with a reduction in the protein synthesis marker leucine, the citric acid cycle intermediate succinic acid, the glycolysis associated metabolites, alanine and phenylacetic acid, and dysregulation of urine volume, lysine metabolism and amino acid excretion. These changes are the same as those noted for alterations in many of the measures indicative of pain severity, distribution and duration noted in the TMD patients in this chapter.

3.4.2.q. Neutrophil count associations with symptoms and biochemical parameters.

A reduction in the neutrophil count was an important factor associated with 12-month facial pain expression and has been reported to be associated with prolonged muscle pain and damage (Bruunsgaard et al, 1997). The neutrophil count was assessed and compared by multi- and uni-variant analyses to identify the associations with symptom expression (Table 3.23) and with any differences associated with the 7-day facial pain severity score in the CFS patients (Table 3.24). Table 3.23 shows that the increase in the neutrophil count was associated with increases in the 7-day severity of cognitive changes, panic attacks and muscle symptoms as well as depression-associated symptoms (Q26, Blaming your self for things and Q89, Feelings of guilt). The increase in the neutrophil count was also associated with increases in the 12-month frequency and severity of fatigue, cognitive symptoms, musculoskeletal symptoms (including facial pain), hair loss, symptoms of diarrhoea and depressive symptoms as assessed in the RDC/TMD Axis II criteria.

Table 3.24 shows that the increase in neutrophil levels in the facial pain positive subjects was associated with increased vitamin B12 levels, Ph2a, leukocyte and monocyte count and reductions in serum phosphate and the urinary relative abundance of threonine and hydroxyproline. In the CFS patients without a 7-day facial pain response increases in the neutrophil count correlated with 15 different measures, whilst differences in correlations were found for 9 different measures.

Table 3.23. Summary of the correlation analyses of the association between the SCL-90-R scalar symptom responses and neutrophil count in the CFS patients.

SCL-90-R Question	7-Day Severity	12-month Frequency	12-month Severity
NEUTROPHIL COUNT	r *P*	r *P*	r *P*
Q45 Having to check and double check what you are doing	0.24 <0.03	0.34 <0.001	0.36 <0.001
Q38 Having to do things slowly to ensure correctness	0.25 <0.02	0.30 <0.005	-
Q42 Soreness of your muscles	0.23 <0.03	0.31 <0.003	-
Q58 Heavy feelings in your arms or legs	0.21 <0.05	0.22 <0.04	-
Q73 Feeling uncomfortable about eating/ drinking in public	-	0.28 <0.009	0.27 <0.02
Q111 Hair loss	-	0.25 <0.02	0.25 <0.02
Q33 Feeling fearful	-	0.24 <0.03	0.24 <0.03
Q100 Confusion	-	0.23 <0.04	0.22 <0.04
Q95 Diarrhoea	-	0.22 <0.05	0.22 <0.05
Q123 Facial pain or discomfort	-	0.21 <0.05	0.22 <0.04
Q72 Spells of terror or panic	0.30 <0.004	-	-
Q89 Feelings of guilt	0.29 <0.006	-	-
Q121 Stomach cramps	0.27 <0.03	-	-
Q26 Blaming yourself for things	0.22 <0.04	-	-
Q51 Having to avoid things, places or activities because they frighten you	0.21 <0.05	-	-
Q2 Nervousness or shakiness inside	-	0.25 <0.02	-
Q14 Feeling low in energy or run down	-	0.24 <0.04	-
Q11 Feeling easily annoyed or irritated	-	0.23 <0.03	-
Q57 Feeling tense or keyed up	-	0.23 <0.04	-
Q106 Fatigue	-	0.21 <0.05	-
Q94 Depression	-	-	0.22 <0.04

7-day Severity regression model; R^2=0.705, F(10,44)=10.512, *P*<0.00000
Variables; 1) Spells of terror or panic *P*<0.01, 2) Stomach cramps *P*<0.02, 3) Blurry vision *P*<0.006
12-Month Frequency regression model; R^2=0.938, F=21.348, *P*<0.00000
Variables; 1) Uncomfortable about eating *P*<0.000001, 2)Blaming yourself -*P*<0.000001, 3) Diarrhoea *P*<0.000001
12-Month Severity regression model; R^2=0.900, F=12.165, *P*<0.00000
Variables; 1) Uncomfortable about eating *P*<0.05, 2) Sore glands -*P*<0.000003, 3) Thoughts not your own -*P*<0.48

Eleven metabolites were statistically different between the facial pain patients and those CFS patients without facial pain. Large differences were found for 2 urinary metabolites (UM28 and threonine) and serum vitamin B12. Thus the increase in the neutrophil count in the facial pain patients was associated with large differences in associations with many different metabolites and immune parameters indicating that the neutrophil count in the facial pain patients was associated with a dysregulation of metabolism. These data confirm the findings that increases in the 12-month frequency and severity of facial pain are associated with increases in the neutrophil count and that these factors are associated with alterations in vitamin B12, serum phosphate and alterations in connective tissue amino acids proline and hydroxyproline.

Table 3.24. Summary of the differences in correlation coefficients for the neutrophil count between CFS patients with a positive 7-day facial pain response compared with those with no response.

Parameter	Facial Pain		No Facial pain		Difference
Increased	r	*P*	r	*P*	
Leukocyte count	0.84	<0.001	0.88	<0.001	NS
Vitamin B12	0.78	<0.008	0.12	NS	<0.008
Serum Ph2a	0.61	<0.02	0.01	NS	<0.05
Monocyte count	0.58	<0.03	0.53	<0.001	NS
% U UM28	0.51	NS	-0.35	<0.02	<0.008
% U Hippuric acid	0.42	NS	-0.37	<0.009	<0.02
Basophil count	0.38	NS	0.44	<0.001	NS
Eosinophil count	0.22	NS	0.29	<0.04	NS
Serum Ph2	0.21	NS	-0.32	<0.03	NS
Platelet count	0.05	NS	0.49	<0.001	NS
Decreased					
% U Lysine	-0.15	NS	0.31	<0.04	NS
% U Glycine	-0.24	NS	0.30	<0.04	NS
% U UM14	-0.27	NS	0.30	<0.04	NS
Serum Phenylalanine	-0.31	NS	0.30	<0.04	NS
% U UM13a	-0.38	NS	0.30	<0.04	<0.04
U % & EPM Proline	-0.46	NS	0.19	NS	<0.05
U % & EPM Threonine	-0.61	<0.03	0.35	<0.02	<0.003
% U Hydroxyproline	-0.63	<0.02	0.06	NS	<0.02
Serum Phosphate	-0.61	<0.02	0.02	NS	<0.04
Serum β-aminoisobutyrate	0.28	NS	-0.34	<0.02	NS
Facial Pain regression model; R^2=0.999, F=44693, *P*<0.00000					
Variables; 1) Leukocyte count *P*<0.000002, 2) % lymphocytes -*P*<0.000004, 3) ESR *P*<0.000006					
No Facial Pain regression model; R^2=0.999, F=8468.2, *P*<0.00000					
Variables; 1) Leukocyte count *P*<0.000001, 2) % lymphocytes -*P*<0.000001, 3) eosinophil count -*P*<0.00002					

3.4.2.r. Associations between Basophil Count Symptoms and Biochemical Parameters.

Tables 3.17 and 3.18 show that the variation in 12-month severity and frequency of facial pain were positively associated with an increase in the basophil count suggesting that histamine based immune cell products may also be involved in the facial pain process as suggested by several investigators (Broton & Sessle, 1988; Broton et al, 1988; Hu et al, 1993; Yu et al, 1995; Yu et al, 1996; Chiang et al, 1997). The multi- and univariate statistical analyses of the associations between the basophil count and symptoms and the biochemical parameters are shown in Tables 3.25 and 3.26, respectively.

Table 3.25 shows that increases in the basophil count in the CFS patients were associated with increases in the 7-day symptom severity of bladder infections and sleep disturbance and the 12-month frequency/ severity of facial muscle and TMJ pain, muscle soreness, diarrhoea and hair loss. The previously reported pilot study association between facial pain and bladder infections/cystitis was noted (McGregor et al, 1992). Thus increases in the basophil count are highly associated with TMD symptoms and a segment of those symptoms reported by TMD patients.

Table 3.25. Summary of the correlation analyses of the association between the SCL-90-R scalar symptom responses and the basophil count.

SCL-90-R Question	7-Day Severity	12-month Frequency	12-month Severity
	r P	r P	r P
Q97 Bladder infections/cystitis	0.26 <0.02	-	-
Q80 Feeling that something bad is going to happen	0.24 <0.02	-	-
Q31 Worrying too much about things	0.21 <0.05	0.25 <0.02	-
Q66 Sleep that is restless or disturbed	0.21 <0.05	-	-
Q123 Facial pain or discomfort	-	0.28 <0.009	0.33 <0.002
Q42 Soreness of your muscles	-	0.26 <0.02	0.22 <0.04
Q111 Hair loss	-	0.25 <0.02	0.27 <0.02
Q95 Diarrhoea	-	0.27 <0.02	-
Q33 Feeling fearful	-	0.24 <0.03	-
Q59 Thoughts of death or dying	-	0.24 <0.03	-
Q105 Jaw joint pain or discomfort	-	0.23 <0.04	-
Q64 Awakening in the early morning		-	0.25 <0.02

7-day Severity regression model; R^2=0.791, F(12,42)=13.262, P<0.00000
Variables; 1) Hair loss P<0.03, 2) Frightening thoughts or images P<0.04, 3) Heartburn/gastric reflux P<0.04
12-Month Frequency regression model; R^2=0.953, F=28.489, P<0.00000
Variables; 1) Bladder infections/cystitis P<0.000001, 2) Feeling fearful -P<0.75, 3) Thoughts of death or dying -P<0.00002
12-Month Severity regression model; R^2=0.976, F=40.973, P<0.00000
Variables; 1) Facial pain P<0.0006, 2) Recurrent genital infections P<0.78, 3) Stomach cramps P<0.02

Table 3.26 shows the association between the basophil count and the biochemical parameters within the CFS patients. In the facial pain patients the basophil count was positively associated with Deildrin, urine volume, total serum amino acid levels and serum s-methylcysteine. In the facial pain patients the basophil count was negatively associated with urinary levels of the first 2 components of the citric acid cycle, citric and aconitic acids. Six different metabolites had statistically different correlation coefficients between the facial pain and no facial pain groups, urinary metabolite excretion per minute, urinary organic acid excretion per minute, urinary tyrosine, aspartic, aconitic and citric acid excretion rates. The pattern of metabolites associated with the basophil count in the facial pain patients was quite different from that seen in the CFS patients with no facial pain. The pesticide Deildrin had a high correlation with the basophil count in the facial pain patients and was also the primary factor determined by multivariate analysis. Thus the basophil count was associated with alterations in a number of nitric oxide mediated events (increased diuresis), alteration in citric acid cycle activity as well as increases in the levels of the organochlorine, Deildrin.

Table 3.26. Summary of the univariate analyses of the association between the basophil count and the biochemical parameters in the CFS patient group and control subjects.

Parameter	Facial Pain		No Facial Pain		Difference
Increased	r	*P*	r	*P*	
Deildrin	0.72	<0.003	0.09	NS	NS
Urine volume	0.57	<0.04	0.08	NS	NS
Total serum amino acids	0.54	<0.05	-0.19	NS	NS
Serum S-methylcysteine	0.53	<0.05	0.23	NS	NS
Leukocyte count	0.38	NS	0.63	<0.001	NS
Neutrophil count	0.38	NS	0.44	<0.001	NS
% Urinary Acetaminophen	0.33	NS	0.34	<0.02	NS
Monocyte count	0.32	NS	0.58	<0.001	NS
% Urinary CFSUM1	0.17	NS	-0.33	<0.02	NS
Eosinophil count	0.05	NS	0.51	<0.001	NS
Lymphocyte count	0.03	NS	0.50	<0.001	NS
Thyroid stimulating hormone	0.02	NS	0.42	<0.003	NS
Platelet count	-0.06	NS	0.31	<0.03	NS
Urinary EPM UM13a	-0.11	NS	0.34	<0.02	NS
Urinary EPM S-methylcysteine	-0.11	NS	0.29	<0.05	NS
Urinary EPM proline	-0.15	NS	0.36	<0.009	NS
Urinary EPM glutamic acid	-0.28	NS	0.36	<0.01	NS
Urinary EPM phenylalanine	-0.30	NS	0.34	<0.02	NS
Urinary EPM UM15a	-0.37	NS	0.38	<0.006	NS
MCV	-0.43	NS	0.29	<0.04	NS
Urinary metabolite/minute	-0.50	NS	0.24	NS	<0.03
Urinary EPM tyrosine	-0.51	NS	0.25	NS	<0.03
Urinary organic acids/minute	-0.52	NS	0.20	NS	<0.04
Urinary EPM aspartic acid	-0.52	NS	0.29	<0.04	<0.02
Decreased					
Red cell count	-0.22	NS	-0.31	<0.03	NS
Urinary EPM Aconitic acid	-0.56	<0.04	0.28	NS	<0.02
% and EPM Urinary Citric acid	-0.74	<0.003	0.11	NS	<0.006
Facial Pain regression model; R^2=0.525, F=6.637, *P*<0.05					
Variables; 1) Dieldrin *P*<0.04					
No Facial Pain regression model; R^2=0.924, F=4.835, *P*<0.03					
Variables; 1) Leukocyte count *P*<0.003, 2) % U acetaminophen *P*<0.006, 3) TSH *P*<0.02					

3.4.2.s. Association between ALT, Symptoms and Biochemical Parameters.

An increase in ALT was positively associated with the 12-month severity and frequency of facial pain (Table 3.17), which may be the result of a blunted nitric oxide response (Kayashima et al, 1995; Mena et al, 1996; Bruunsgaard et al, 1997). Table 3.25 shows the correlation analyses for serum ALT levels and the 7-day and 12-month severity and frequency symptom responses for the CFS patients. Table 3.27 shows that increasing ALT levels were associated with alterations in TMD, musculoskeletal, sleep disturbance and depression and mood alteration symptoms, including most associated with depression. The questions normally associated with depression, feelings of worthlessness, guilt and the lack of motivation were associated with this tissue damage marker (ALT). This shows that the RDC/TMD Axis II depression assessment is associated with biochemical changes consistent with tissue or liver damage offering a potential biochemical basis for depression.

Table 3.27. Summary of the correlation analyses of the association between the SCL-90-R scalar symptom responses and ALT.

SCL-90-R Question	7-Day Severity	12-month Frequency	12-month Severity
Increased	r P	r P	r P
Q87 Idea that something serious is wrong with your mind	0.27 <0.02	0.24 <0.03	0.24 <0.03
Q72 Spells of terror or panic	0.26 <0.02	0.22 <0.05	0.31 <0.004
Q5 Loss of libido	0.23 <0.04	0.30 <0.006	-
Q24 Temper outbursts that you cannot control	0.23 <0.03	-	0.24 <0.03
Q58 Heavy feelings in your arms or legs	-	0.30 <0.006	0.30 <0.005
Q42 Soreness of your muscles	-	0.29 <0.007	0.31 <0.004
Q123 Facial pain or discomfort	-	0.28 <0.009	0.37 <0.0005
Q80 Feeling that something bad is going to happen	-	0.26 <0.02	0.31 <0.003
Q126 Tooth grinding	0.25 <0.05	-	-
Q79 Feeling of worthlessness	0.24 <0.03	-	-
Q89 Feelings of guilt	-	0.32 <0.003	-
Q110 Frustration	-	0.29 <0.006	-
Q56 Feeling weak in parts of your body	-	0.27 <0.02	-
Q66 Sleep that is restless or disturbed	-	0.24 <0.03	-
Q64 Awakening in the early morning	-	0.22 <0.05	-
Q116 Nightmares	-	-	0.28 <0.009
Q90 Idea that something serious is wrong with your body	-	-	0.26 <0.02
Q70 Feeling uneasy in crowds, such as shopping	-	-	0.25 <0.02
Q50 Having to avoid certain things, places, or activities	-	-	0.25 <0.03
Q71 Feeling everything is an effort	-	-	0.25 <0.03
Q27 Pains in low back	-	-	0.23 <0.04
Q95 Diarrhoea	-	-	0.23 <0.04
Q49 Hot or cold spells	-	-	0.22 <0.04
Q107 Fear of closed spaces	-	-	0.21 <0.05
Decreased			
Q19 Poor appetite	-0.28 <0.008	-0.23 <0.04	-

7-day Severity regression model; R^2=0.736, F(10,44)=12.240, P<0.00000
Variables; 1) Tooth grinding P<0.02, 2) Something wrong mind P<0.03, 3) Trouble remembering things P<0.04
12-Month Frequency regression model; R^2=0.980, F=67.532, P<0.00000
Variables; 1) Facial pain P<0.0003, 2) Feeling blue -P<0.000001, 3)Feeling fearful P<0.000001
12-Month Severity regression model; R^2=0.962, F=31.266, P<0.00000
Variables; 1) Facial Pain $P<4.0E^{-14}$, 2) Feeling lonely -P<0.000002, 3) Repeating actions P<0.000003

Table 3.28 shows the multi- and uni-variant analyses of the association between ALT levels and the biochemical parameters. In all patients ALT levels were associated with AST, urea and urinary glutamic acid levels however in the facial pain patients ALT was associated with increases in the % neutrophils and s-methylcysteine and reductions in serum lysine, the platelet and lymphocyte counts, and the serum tyrosine: leucine ratio. Differences in the correlation coefficients between the facial pain patients and the non facial pain CFS patients were identified for s-methylcysteine, serum glutamic acid, serum lysine and the serum tyrosine leucine ratio. These data show that the increase in ALT levels in the facial pain patients is associated with an increase in the % neutrophils, the urinary excretion of the excitatory amino acid, glutamic acid, and reductions in the urea cycle amino acid lysine even though the level of serum urea is positively associated with ALT.

Table 3.28. Summary of the differences in correlation coefficients for ALT between CFS patients with a positive 7-day facial pain response compared with those with no response.

Parameter	Facial Pain		No Facial pain		Difference
Increased	r	*P*	r	*P*	
AST	0.78	<0.001	0.63	<0.001	NS
Urea	0.73	<0.003	0.31	<0.04	NS
s-Methylcysteine	0.60	<0.03	-0.03	NS	<0.04
% Neutrophils	0.58	<0.03	0.16	NS	NS
% & EPM U Glutamic acid	0.57	<0.04	0.38	<0.007	NS
Creatinine	0.43	NS	0.39	<0.006	NS
Creatine kinase	0.43	NS	0.39	<0.006	NS
GGT	0.39	NS	0.60	<0.001	NS
mls Urine/minute	0.28	NS	0.37	<0.008	NS
% U Aspartic acid	0.27	NS	0.38	<0.008	NS
Serum ethanolamine	0.22	NS	0.29	<0.05	NS
Haematocrit	0.16	NS	0.44	<0.002	NS
Haemoglobin	0.15	NS	0.47	<0.001	NS
DDE	0.05	NS	0.38	<0.009	NS
Red cell count	0.03	NS	0.38	<0.006	NS
% U Lysine	-0.13	NS	0.31	<0.04	NS
Decreased					
U EPM Hippuric acid	-0.06	NS	-0.30	<0.04	NS
Chloride	0.33	NS	-0.31	<0.03	NS
Eosinophil count	0.01	NS	-0.31	<0.04	NS
Serum Serine	-0.23	NS	-0.32	<0.03	NS
% U Phenylalanine	-0.29	NS	0.30	<0.04	NS
Serum Phosphate	-0.42	NS	-0.39	<0.008	NS
Serum Glutamic acid	-0.51	NS	0.13	NS	<0.05
Serum Lysine	-0.54	<0.05	0.13	NS	<0.04
Platelet count	-0.55	<0.05	0.05	NS	NS
Serum Tyrosine: leucine	-0.56	<0.04	0.08	NS	<0.04
Lymphocyte count	-0.58	<0.04	-0.18	NS	NS
Facial Pain regression model; R^2=0.946, F=23.248, *P*<0.006					
Variables; 1) AST *P*<0.003, 2) Serum Tryptophan *P*<0.01, 3) serum lysine -*P*<0.03					
No Facial Pain regression model; R^2=0.979, F=34.415, *P*<0.00000					
Variables; 1) AST *P*<0.000004, 2) GGT *P*<0.00005, 3) Serum serine -*P*<0.0006					

3.4.2.t. Association between the Excitatory Amino Acids, Symptoms and Biochemical Parameters.

As the excitatory amino acids, aspartic and glutamic acid, were identified to be associated with increasing pain severity in the RDC/TMD type 1a group they were assessed to establish the relationship with symptoms and biochemical parameters by multi- and uni-variant analysis. Table 3.29 shows that the excretion rate per minute of aspartic acid was positively associated with the 7-day responses to allergies, rashes and nightmares and the 12-month frequency and severity of facial pain, headaches, weakness and 3 psychological responses. The excretion rate per minute for glutamine/glutamic acid was only associated with 3 symptoms. Multi-variant analyses identified primary associations between increasing excitatory amino acids and alterations in mood or psychological responses.

Table 3.29. Summary of the correlation analyses of the association between the SCL-90-R scalar symptom responses and the EPM for glutamic and aspartic acids.

SCL-90-R Question	7-Day Severity	12-month Frequency	12-month Severity
Aspartic acid Increased	r *P*	r *P*	r *P*
Q92 Allergies	0.28 <0.03	-	-
Q116 Nightmares	0.28 <0.03	-	-
Q119 Rashes	0.27 <0.03	-	-
Q10 Worried about sloppiness or carelessness	-	0.25 <0.02	-
Q68 Having ideas or beliefs that others do not share	-	0.24 <0.03	-
Q36 Feeling others don't understand you or are unsympathetic	-	0.23 <0.03	-
Q56 Feeling weak in parts of your body	-	0.23 <0.03	-
Q1 Headaches	-	-	0.26 <0.01
Q123 Facial pain or discomfort	-	-	0.23 <0.04
Glutamic acid Increased			
Q116 Nightmares	0.29 <0.02	-	-
Q1 Headaches	-	-	0.24 <0.03
Decreased			
Q47 Feeling afraid to travel on buses, subways or trains	-0.27 <0.009	-	-

Aspartic acid
7-day Severity regression model; $R^2=0.869$, $F(16,38)=15.815$, $P<0.00000$
Variables; 1) Nightmares $P<0.000001$, 2) Sore throat $P<0.0001$, 3) Constipation $P<0.000001$
12-Month Frequency regression model; $R^2=0.972$, $F=43.936$, $P<0.00000$
Variables; 1) Feelings of worthlessness $P<0.0000001$, 2) Poor circulation $P<0.000001$, 3) Joint pain $P<0.00003$
12-Month Severity regression model; $R^2=0.956$, $F=27.026$, $P<0.00000$
Variables; 1) Uneasy in crowds $P<0.00000003$, 2) Headaches $P<0.14$, 3) Paranoia -$P<0.000001$

Table 3.30 shows that the increases in the excretion rate per minute of both aspartic acid and glutamine/glutamic acid are highly correlated with the excretion of many metabolite as well as the total urinary metabolites and total urinary amino acids and therefore reflect the increased excretion of metabolite. Twelve biochemical parameters had different correlation coefficients between the facial pain and no facial pain CFS patients. These include the urine volume, vitamin B12, DDE, alanine, asparagine, aconitic acid and several leukocyte parameters (basophils, monocytes and lymphocytes). The changes in metabolites associated with increases in the excretion rate per minute of aspartic acid were predominantly associated with the increase in metabolites associated with increasing urine volume. This is very similar to that noted in the RDC/TMD type 1a group analysis.

Table 3.30. Summary of the differences in correlation coefficients for aspartic acid between CFS patients with a positive 7-day facial pain response compared with those with no response.

Parameter	**Facial Pain**		**No Facial pain**		**Difference**
	r	*P*	r	*P*	
Increased					
% & EPM U UM15A	0.90	<0.001	0.66	<0.001	<0.05
% & EPM U Glutamic acid	0.89	<0.001	0.89	<0.001	NS
Total Urinary Amino acids	0.88	<0.001	0.87	<0.001	NS
EPM U Ethanolamine	0.87	<0.001	0.63	<0.001	NS
Total Urinary Metabolite	0.87	<0.001	0.85	<0.001	NS
EPM U Threonine	0.86	<0.001	0.77	<0.001	NS
EPM U 3-Methylhistidine	0.85	<0.001	0.62	<0.001	NS
% & EPM U Aconitic acid	0.85	<0.001	0.40	<0.004	<0.02
EPM U Ornithine	0.84	<0.001	0.66	<0.001	NS
EPM U Hydroxyproline	0.83	<0.001	0.71	<0.001	NS
EPM U Tyrosine	0.82	<0.001	0.60	<0.001	NS
EPM U Lysine	0.82	<0.001	0.66	<0.001	NS
EPM U Glycine	0.81	<0.001	0.70	<0.001	NS
EPM U Serine	0.78	<0.001	0.69	<0.001	NS
EPM U UM14	0.78	<0.001	0.28	<0.05	<0.03
EPM U Valine	0.75	<0.002	0.76	<0.001	NS
EPM U Phenylalanine	0.74	<0.003	0.70	<0.001	NS
EPM U Alanine	0.74	<0.003	0.73	<0.001	NS
Total Urinary Organic acids	0.72	<0.004	0.58	<0.001	NS
EPM U Proline	0.71	<0.004	0.74	<0.001	NS
EPM U Leucine	0.71	<0.004	0.61	<0.001	NS
Serum Alanine	0.70	<0.005	0.01	NS	<0.02
EPM U β-Alanine	0.70	<0.006	0.62	<0.001	NS
EPM U UM17	0.69	<0.006	0.22	NS	NS
EPM U S-methylcysteine	0.67	<0.009	0.31	<0.03	NS
Serum Asparagine	0.67	<0.009	-0.20	NS	<0.004
EPM U CFSUM1	0.66	<0.01	0.15	NS	NS
EPM U UM28	0.66	<0.01	0.15	NS	NS
EPM U UM13	0.64	<0.02	0.20	NS	NS
EPM U UM13a	0.63	<0.02	0.38	<0.007	NS
EPM U Citric acid	0.62	<0.02	0.45	<0.001	NS
EPM U UM15	0.59	<0.03	0.33	<0.02	NS
EPM U Succinic acid	0.53	NS	0.40	<0.004	NS
EPM U β-aminoisobutyrate	0.49	NS	0.42	<0.003	NS
EPM U Phenylacetic acid	0.46	NS	0.36	<0.01	NS
EPM U UM27	0.42	NS	0.34	<0.02	NS
Urinary Tyrosine: leucine	0.31	NS	0.43	<0.002	NS
Red cell distribution width	0.04	NS	0.39	<0.005	NS
Decreased					
Free Thyroid T4 hormone	-0.01	NS	-0.28	<0.05	NS
Urine volume	-0.12	NS	0.54	<0.001	<0.04
Vitamin B12	-0.28	NS	0.37	<0.05	<0.05
DDE	-0.46	NS	0.23	NS	<0.04
Leukocyte count	-0.47	NS	0.21	NS	<0.04
Monocyte count	-0.51	NS	0.33	<0.02	<0.01
Lymphocyte count	-0.52	NS	0.12	NS	<0.05
Basophil count	-0.52	NS	0.29	<0.05	<0.02

Facial Pain regression model:
Variables: 1) P<0.000001, 2) P<0.000001, 3) P<0.000001
No Facial Pain regression model:
Variables: 1) P<0.000001, 2) P<0.000004, 3) P<0.00002

3.4.2.u. Association between Serum Lysine, Symptoms and Biochemical Parameters.

Lysine levels were found to be the major parameter associated with alterations in the metabolites excreted independent of changes in the urine volume (Tables 3.11 and 3.12). As lysine is an amino acid that competes with arginine uptake and may therefore alter nitric oxide production, the association between serum lysine and symptoms and biochemical parameters was assessed by multi- and uni-variant analyses.

Table 3.31. Summary of the correlation analyses of the association between the SCL-90-R scalar symptom responses and serum lysine.

SCL-90-R Question	**7-Day Severity**	**12-month Frequency**	**12-month Severity**
Increased	r *P*	r *P*	r *P*
Q105 Jaw joint pain or tenderness	-	0.29 <0.007	-
Q40 Nausea or upset stomach	-	0.24 <0.02	-
Decreased			
Q65 Having to repeat the same actions such as touching	-0.25 <0.02	-0.27 <0.01	-0.26 <0.02
Q69 Feeling very self-conscious with others	-0.25 <0.02	-0.21 <0.05	-
Q21 Feeling shy or uneasy with the opposite sex	-0.23 <0.03	-0.25 <0.02	-
Q70 Feeling uneasy in crowds, such as shopping	-0.23 <0.03	-0.26 <0.02	-
Q59 Thoughts of death or dying	-	-0.28 <0.01	-0.25 <0.02
Q112 Heartburn / Gastric reflux	-	-0.24 <0.03	-0.22 <0.04
Q124 Swallowing difficulties	-0.40 <0.001	-	-
Q41 Feeling inferior to others	-0.28 <0.007	-	-
Q106 Fatigue	-0.30 <0.02	-	-
Q73 Feeling uncomfortable about eating or drinking in public	-0.25 <0.02	-	-
Q100 Confusion	-0.26 <0.04	-	-
Q103 Sore glands	-0.26 <0.04	-	-
Q92 Allergies	-0.25 <0.04	-	-
Q10 Worried about sloppiness or carelessness	-0.21 <0.04	-	-
Q115 Muscle twitches	-0.24 <0.05	-	-
Q116 Nightmares	-0.25 <0.05	-	-
Q4 Faintness or dizziness	-	-0.29 <0.007	-
Q2 Nervousness or shakiness inside	-	-0.25 <0.02	-
Q25 Feeling afraid to go out of your house alone	-	-0.26 <0.02	-
Q28 Feeling blocked in getting things done	-	-0.24 <0.03	-
Q47 Feeling afraid to travel on buses, subways or trains	-	-0.23 <0.03	-
Q45 Having to check and double-check what you do	-	-	-0.29 <0.007
Q38 Having to do things very slowly to ensure correctness	-	-	-0.26 <0.02
Q55 Trouble concentrating	-	-	-0.21 <0.05
7-day Severity regression model; R^2=0.914, F=13.762, P<0.00000			
Variables; 1) Nightmares -P<0.0000004, 2) Bladder infections/cystitis P<0.000002, 3) Frightening thoughts or images P<0.000005			
12-Month Frequency regression model; R^2=0.964, F=37.378, P<0.00000			
Variables; 1) Heartburn/gastric reflux -P<0.000001, 2) Nausea P<0.000001, 3) Faintness/dizziness -P<0.000001			
12-Month Severity regression model; R^2=0.930, F=17.833, P<0.00000			
Variables; 1) Someone else controlling thoughts -P<0.000001, 2) Abdominal pain P<0.000001, 3) Heartburn/gastric reflux -P<0.0001			

Table 3.31 shows that the increase in serum lysine is associated with TMJ pain and nausea. However, falls in serum lysine levels were associated with fatigue, psychological, cognitive and musculoskeletal symptoms. Table 3.31 shows that within the facial pain

patients serum lysine was positive associated with serum ornithine, serum aconitic acid and serum tryptophan and negatively associated with sodium, serum glycine, vitamin B12 and ALT. Correlation coefficient differences between the facial pain and no facial pain patients were identified for 9 parameters. These included sodium, ALT, vitamin B12, platelet count and volume, serum tyrosine, creatinine, urea and the urinary excretion rate per minute of β-alanine.

Table 3.32. Summary of the differences in correlation coefficients for serum lysine between CFS patients with a positive 7-day facial pain response compared with those with no response.

Parameter	**Facial Pain**		**No Facial pain**		**Difference**
Increased	r	P	r	P	
Serum Ornithine	0.65	<0.009	0.43	<0.002	NS
Serum Aconitic acid	0.57	<0.03	0.18	NS	NS
Serum Tryptophan	0.53	<0.05	0.45	<0.001	NS
Serum Platelet count	0.48	NS	-0.22	NS	<0.03
Serum Tyrosine: leucine	0.48	NS	0.60	<0.001	NS
Serum Glutamic acid	0.44	NS	0.69	<0.001	NS
Total serum amino acids	0.18	NS	0.52	<0.001	NS
Iron	-0.02	NS	0.40	<0.006	NS
Serum Phenylalanine	-0.07	NS	0.40	<0.005	NS
Serum Tyrosine	-0.25	NS	0.46	<0.001	<0.03
Mean platelet volume	-0.36	NS	0.29	<0.05	<0.05
EPM U β-Alanine	-0.44	NS	0.24	NS	<0.04
Creatinine	-0.47	NS	0.21	NS	<0.03
Urea	-0.53	NS	0.19	NS	<0.02
Decreased					
Serum β-Aminohydroxyproprionate	0.25	NS	-0.36	<0.01	NS
ALP	0.20	NS	-0.30	<0.04	NS
Serum Ethanolamine	0.19	NS	-0.30	<0.04	NS
% U Aconitic acid	0.13	NS	-0.41	<0.003	NS
Serum Ph1	0	NS	-0.33	<0.03	NS
Serum Ph2	-0.05	NS	-0.42	<0.003	NS
Serum Ph2a	-0.07	NS	-0.30	<0.04	NS
% U Glutamic acid	-0.13	NS	-0.33	<0.03	NS
Serum Threonine	-0.22	NS	-0.47	<0.001	NS
Serum Valine	-0.23	NS	-0.54	<0.001	NS
Serum Isoleucine	-0.32	NS	-0.59	<0.001	NS
Serum Leucine	-0.43	NS	-0.57	<0.001	NS
Serum Alanine	-0.43	NS	-0.52	<0.001	NS
ALT	-0.54	<0.05	0.13	NS	<0.03
Vitamin B12	-0.55	<0.05	0.15	NS	<0.03
Serum Glycine	-0.57	<0.03	-0.55	<0.001	NS
Sodium	-0.61	<0.02	-0.04	NS	<0.05

Facial Pain regression model: R^2=0.998, F=152.39, P<0.0001
Variables: 1) Serum Glutamic acid P<0.00007, 2) Serum Ornithine P<0.00002, 3) CFSUM1 P<0.03
No Facial Pain regression model: R^2=0.979, F=53.171, P<0.00000
Variables: 1) Serum Tryptophan P<0.000001, 2) Serum asparagine P<0.00003, 3) % U Phenylacetic acid P<0.000001

To assess if there was any dysregulation in the amino acids associated with membrane arginine uptake and nitric oxide production the serum lysine: ornithine ratio was calculated.

The lysine: ornithine ratio was increased in the CFS group compares with the control group (CFS = 1.01±0.38; Control = 0.90±0.44 - P<0.05). In the CFS group the lysine: ornithine ratio was positively correlated with Q14 (Feeling low in energy or run down; r=0.27, P<0.009), Q30 (Feeling blue), Q66 (Restless or disturbed sleep; both r=0.24, P<0.02), Q71 (Feeling that everything is an effort), Q22 (Feelings or being trapped or caught), Q86 (Thoughts or images of a frightening nature; all 3 r=0.22, P<0.04) and Q82 (Feeling afraid you will faint in public; r=0.21, P<0.05) and negatively correlated with Q95 (Diarrhoea; r=-0.32, P<0.01). In the CFS group the lysine: ornithine ratio was positively correlated with serum glutamine/glutamic acid (r=0.47, P<0.001), tyrosine (r=0.30, P<0.002), tryptophan, β-alanine (both r=0.25, P<0.02), phenylalanine and aconitic acid (both r=0.23, P<0.03), and negatively correlated with the branched chain amino acids valine and isoleucine (both r=-0.29, P<0.004), leucine and alanine (r=-0.23, P<0.03).

The increases in serum lysine in the facial pain CFS patients are associated with increases in TMJ pain and falls in sodium and the tissue damage marker, ALT. Also of importance is that serum lysine is positively associated with the serotonin precursor, tryptophan, and negatively associated with many psychological responses associated with clinical depression, such as fatigue and thoughts of death or dying, and cognitive dysfunction. Thus lysine is associated with changes in TMD symptom expression and biochemistry.

3.4.2.v. Association between urine volume, Symptoms and Biochemical Parameters.

Alterations in urine volume were found to be a major parameter associated with TMD pain expression in the CFS group and the symptom (Table 3.33) and potential biochemical (Table 3.34) basis were investigated using multi- and uni-variant analyses. In the 7-day severity analysis increasing urine volume was associated with weakness was the primary regression variable whilst in the 12-month frequency and severity regression models urine volume was associated with the primary symptoms of rashes and nightmares, respectively. Uni-variant correlation analysis showed that increasing urine volume was associated principally with musculoskeletal symptoms (16 symptoms) and psychological responses (12 symptoms).

Table 3.34 summarises the biochemical parameter associations with increasing urine volume in the CFS patients with a positive 7-day face pain response and the remaining CFS patients. In the patients with face pain the primary multiple regression variable for increasing

Table 3.33. Summary of the correlation analyses of the association between the SCL-90-R scalar symptom responses and urine volume.

SCL-90-R Question	7-Day Severity	12-month Frequency	12-month Severity
Increased	r *P*	r *P*	r *P*
Q42 Soreness of your muscles	0.24 <0.03	0.28 <0.008	0.29 <0.007
Q56 Feeling weak in parts of your body	0.29 <0.006	0.24 <0.03	-
Q14 Feeling low in energy or run down	0.22 <0.04	-	0.26 <0.02
Q12 Pains in heart or chest	0.21 <0.05	-	0.22 <0.04
Q123 Facial pain or tenderness	-	0.27 <0.02	0.27 <0.02
Q72 Spells of terror or panic	-	0.24 <0.03	0.25 <0.03
Q113 Joint pain	-	0.24 <0.03	0.26 <0.02
Q5 Loss sexual interest or pleasure	-	0.24 <0.03	0.23 <0.04
Q124 Swallowing difficulties	-	0.22 <0.05	0.22 <0.05
Q71 Feeling uneasy in crowds, shopping or at the movies	-	0.22 <0.05	0.23 <0.04
Q58 Heavy feelings in your arms and legs	0.30 <0.004	-	-
Q15 Thoughts of ending your life	0.28 <0.008	-	-
Q60 Overeating	0.28 <0.009	-	-
Q39 Heart pounding or racing	0.27<0 .02	-	-
Q82 Feeling afraid you will faint in public	0.26 <0.02	-	-
Q52 Numbness or tingling in parts of your body	0.26 <0.02	-	-
Q105 Jaw joint pain or discomfort	0.27 <0.03	-	-
Q17 Trembling	0.24 <0.03	-	-
Q13 Feeling afraid in open spaces or on the streets	0.23 <0.03	-	-
Q33 Feeling fearful	0.23 <0.03	-	-
Q57 Feeling tense or keyed up	0.23 <0.04	-	-
Q7 The idea that someone else can control your thoughts	0.22 <0.04	-	-
Q80 Feeling something bad is going to happen to you	0.21 <0.05	-	-
Q2 Nervousness or shakiness inside	0.21 <0.05	-	-
Q116 Nightmares	-	-	0.34 <0.001
Q1 Headaches	-	-	0.24 <0.03
Q28 Feeling blocked in getting things done	-	-	0.24 <0.03
Q106 Fatigue	-	-	0.22 <0.04

7-day Severity regression model; R^2=0.931, F=17.472, *P*<0.00000
Variables; 1) Weak in parts of body *P*<0.000002, 2) Someone controls your thoughts *P*<0.000003, 3) Frequent arguments -*P*<0.43
12-Month Frequency regression model; R^2=0.943, F=21.514, *P*<0.00000
Variables; 1) Rashes -*P*<0.009, 2) Uneasy about people watching or talking about you -*P*<0.000001, 3) Spells of terror or panic *P*<0.000001
12-Month Severity regression model; R^2=0.878, F=8.984, *P*<0.00000
Variables; 1) Nightmares *P*<0.0002, 2) Easily annoyed or irritated -*P*<0.93, 3) Hopeless about future -*P*<0.72

urine volume was a reduction in the serum protein levels whilst in the remaining CFS patients with no face pain the primary discriminant variable was the relative abundance of urinary lysine. Fifteen of the 36 urinary metabolites were significantly positively correlated with urine volume in the CFS group with no facial pain (Data in Table 3.11) and only aspartic acid had a statistically significant different correlation between the facial pain and remaining CFS patient groups (Urine volume/aspartic acid: Facial pain=-0.12 *P*=NS; No facial pain=0.55 <0.001; difference *P*<0.04). Uni-variant analysis of the biochemical parameter associations with increasing urine volume in the facial pain patients revealed that increasing urine volume was associated with increases in the urinary relative abundance of serine, β-aminoisobutyrate and UM13, serum unknown Ph1 and the basophil count in the facial pain group. Increasing urine volume was negatively correlated with the urinary relative abundance of citric acid, 1-methylhistidine and unknown UM27, serum protein and albumin levels. Ten parameters had statistically different correlations with urine volume in the facial pain group compared with the remaining CFS group. Thus alteration in the basophil count along with lower albumin and serum protein levels are associated with variation in urine volume in the CFS patients reporting facial pain.

Table 3.34. Summary of the differences in correlation coefficients for urine volume between CFS patients with a positive 7-day facial pain response compared with those with no response.

Parameter	Facial Pain		No Facial pain		Difference
	r	*P*	r	*P*	
Increased					
% U Serine	0.61	<0.02	-0.21	NS	<0.008
% U β-aminoisobutyrate	0.58	<0.03	-0.23	NS	<0.01
Basophil count	0.57	<0.04	0.07	NS	NS
Serum Ph1	0.56	<0.04	0.09	NS	NS
% U UM13	0.54	<0.05	-0.15	NS	<0.03
% U Succinic acid	0.39	NS	-0.30	<0.04	<0.04
Sodium	0.37	NS	-0.35	<0.02	<0.03
ALT	0.28	NS	0.38	<0.008	NS
Serum Alanine	0.23	NS	0.31	<0.04	NS
% U UM17	0.17	NS	-0.30	<0.04	NS
Red blood cell distribution width	0.16	NS	0.34	<0.02	NS
Serum Ph2	0.11	NS	0.32	<0.03	NS
Serum Proline	0.09	NS	-0.35	<0.02	NS
% Monocytes	0.02	NS	0.29	<0.04	NS
Decreased					
Serum ethanolamine	-0.01	NS	0.28	<0.05	NS
% U Lysine	-0.03	NS	0.48	<0.001	NS
% U CFSUM1	-0.05	NS	-0.30	<0.04	NS
% U Tyrosine	-0.12	NS	0.31	<0.04	NS
Serum valine	-0.30	NS	0.34	<0.02	NS
% U 1-Methylhistidine	-0.57	<0.04	-0.04	NS	<0.05
% U UM27	-0.65	<0.02	-0.05	NS	<0.04
Serum Albumin	-0.66	<0.02	-0.01	NS	<0.03
% U Citric acid	-0.72	<0.004	-0.04	NS	<0.02
Serum Protein	-0.74	<0.003	-0.14	NS	<0.02

Facial Pain regression model; R^2=0.956, F=16.456, P<0.03
Variables; 1) Serum protein -P<0.006, 2) Serum leucine -P<0.02, 3) Serum tryptophan -P<0.03
No Facial Pain regression model; R^2=0.791, F=6.162, P<0.003
Variables; 1) %U lysine P<0.002, 2) Serum proline -P<0.006, 3) TSH P<0.004

3.4.2.w. DDE and Deildrin associations with symptoms and biochemical parameters.

The organochlorine pesticides DDE and Deildrin were found to be statistically significant parameters in expression of facial pain in the CFS patient group. Thus multi- and uni-variant analyses were undertaken to assess the symptom and biochemical parameters associated with variation in DDE and Deildrin levels in the CFS patients with facial pain and the remaining CFS patients as well as the control group. Table 3.35 shows the multi- and uni-variant analyses for DDE in association with the 7-day severity, and the 12-month severity and frequency of reporting of symptoms.

Table 3.35. Summary of the correlation analyses of the association between the SCL-90-R scalar symptom responses and DDE.

SCL-90-R Question	**7-Day Severity**	**12-month Frequency**	**12-month Severity**
CFS Patients	r *P*	r *P*	r *P*
Increased			
Q122 Earaches	-	0.26 <0.02	0.31 <0.004
Q123 Facial pain	-	0.22 <0.04	0.22 <0.05
Q5 Loss of sexual interest or pleasure	-	0.24 <0.03	-
Q64 Awakening early in the morning	-	0.24 <0.03	-
Q124 Swallowing difficulties	-	0.23 <0.04	-
Q59 Thoughts of death or dying	-	-	0.27 <0.01
Decreased			
Q36 Feeling others do not understand or are unsympathetic	-0.29 <0.006	-	-0.25 <0.02
Q37 Feeling that people are unfriendly or dislike you	-0.24 <0.03	-	-0.22 <0.04
Q93 Constipation	-0.30 <0.02	-	-
Q99 Burning urination	-0.26 <0.05	-	-
Q81 Shouting or throwing things	-0.23 <0.03	-	-
Q85 The idea you are being punished for your sins	-0.22 <0.04	-	-
Q44 Trouble falling asleep	-	-0.24 <0.03	-
Q94 Depression	-	-0.22 <0.04	-
Control Subjects			
Increased			
Q119 Rashes	0.34 <0.03	-	-
Q60 Overeating	-	-	0.24 <0.04
Q5 Loss of sexual interest or pleasure	-	-	0.23 <0.05
Decreased			
Q12 Pains in heart or chest	-	-0.24 <0.04	-0.30 <0.02
Q31 Worrying too much about things	-0.35 <0.005	-	-
Q15 Thoughts of ending your life	-0.33 <0.008	-	-
Q66 Sleep that is restless or disturbed	-0.29 <0.03	-	-
Q76 Others do not give credit for your achievements	-0.26 <0.04	-	-
Q83 People will take advantage if you let them	-0.25 <0.05	-	-
Q67 Having urges to smash or break things	-	-0.26 <0.03	-
Q43 Feeling that you are watched or talked about by others	-	-	-0.28 <0.02
Q61 Feeling uneasy when people are watching or talking about you	-	-	-0.28 <0.02
Q34 Your feelings being easily hurt	-	-	-0.24 <0.04
Q65 Repeating the same actions such as touching, counting or washing	-	-	-0.24 <0.04
Q84 Having thoughts about sex that bother you	-	-	-0.25 <0.04

7-day Severity regression model; $R^2=0.722$, $F(12,42)=9.103$, $P<0.00000$
Variables; 1) Constipation $-P<0.000001$, 2) Heartburn/gastric reflux $P<0.00005$, 3) Poor circulation $P<0.0000001$
12-Month Frequency regression model; $R^2=0.963$, $F=36.133$, $P<0.00000$
Variables; 1) Trouble getting breath $P<0.000001$, 2) Suddenly scared for no reason $-P<0.000001$, 3) Frightening thoughts or images $P<0.000001$
12-Month Severity regression model; $R^2=0.959$, $F=31.058$, $P<0.00000$
Variables; 1) Others unsympathetic $-P<0.02$, 2) Thoughts of death or dying $P<0.000002$, 3) Feeling lonely even when with people $P<0.04$

In the CFS patients multiple regression analysis identified a negative correlation with constipation as the primary symptom associated with DDE levels in the 7-day symptom severity analysis. In the 12-month frequency and severity multiple regression analysis, "trouble getting your breath" and "others being unsympathetic", respectively, were the primary regression questionnaire symptoms associated with DDE.

Table 3.35 shows the uni-variant analyses for the 7-day, 12-month severity and frequency responses in the CFS patients and the control subjects. DDE was also only associated with 12-month severity and frequency of TMD symptoms in the CFS patients but not the control subjects. Table 3.36 shows the multi- and uni-variant analyses of the associations between DDE and the biochemical parameters.

Table 3.36. Summary of the multiple regression and correlation analysis associations between DDE and biochemistry in CFS patients and control subjects.

Parameter	**CFS**	**Control**
Age	0.38 <0.001	0.41 <0.001
Duration	0.21 <0.05	NA
Positive Correlations		
Glucose	0.30 <0.006	NA
Serum S-Methylcysteine	0.29 <0.004	0.09
Serum Ethanolamine	0.27 <0.008	0.27 <0.01
Urine Tyrosine: Leucine	0.26 <0.02	0.26 <0.02
% Urine Acetaminophen	0.25 <0.02	0.04
ALT	0.23 <0.03	NA
Serum Ph2a	0.22 <0.04	0.11
% Basophils	0.20 <0.05	0.01
% Urine Glutamine/Glutamic acid	0.20 <0.05	-0.05
Urine volume	0.18	0.30 <0.008
% Monocytes	0.17	0.28 <0.01
EPM Hippuric acid	-0.08	0.26 <0.02
Total urinary organic acid	0.02	0.24 <0.04
% Urinary Citric acid	0.08	0.24 <0.04
Negative Correlations		
Serum Proline	-0.31 <0.002	-0.03
Serum Serine	-0.28 <0.005	-0.10
% urine UM13	-0.26 <0.02	-0.20
EPM urine CFSUM1	-0.25 <0.02	0.07
EPM urine UM28	-0.25 <0.02	0.07
% urine UM14	-0.20 <0.05	-0.14
% Urine Phenylalanine	-0.02	-0.29 <0.01
% Urine Hydroxyproline	-0.02	-0.27 <0.01
% Urine Tyrosine	-0.12	-0.25 <0.03
% Urine Threonine	-0.12	-0.25 <0.03
% Urine Leucine	-0.15	-0.24 <0.04
Serum Tyrosine: Leucine	-0.06	-0.24 <0.04
Total Serum amino acids	-0.14	-0.24 <0.04
%Urine UM17	-0.18	-0.22 <0.05

CFS regression model; R^2=0.535,F=2.832, P<0.009
Variables; 1) Serum Proline -P<0.01, 2) Serum S-methylcysteine P<0.04, 3) Glucose P<0.07
Control regression model; R^2=0.767, F=9.362, P<0.00000
Variables; 1) Urine volume P<0.00002, 2) %U Threonine -P<0.0004, 3) % Monocytes P<0.000001

NA = Not available

DDE correlated with age in all study subjects and with illness duration in the CFS patients. In the CFS group the primary multiple regression parameter was a reduction in serum proline whilst in the control group the primary discriminant variable was urine volume. In the uni-variant analysis DDE was positively associated with glucose, ALT, % basophils, the urinary tyrosine: leucine ratio, serum ethanolamine, s-methylcysteine and Ph2a, and the urinary relative abundance of acetaminophen and glutamic acid. DDE was negative correlated with serum proline and serine, and the urinary relative abundance of UM13 and UM14 as well as the urinary excretion rate per minute of CFSUM1 and UM28.

Table 3.37. Summary of the correlation analyses of the association between the SCL-90-R scalar symptom responses and Deildrin.

SCL-90-R Question	**7-Day Severity**	**12-month Frequency**	**12-month Severity**
CFS Patients	*r P*	*r P*	*r P*
Q86 Thoughts or images of a frightening nature	0.27 <0.02	0.27 <0.01	0.22 <0.04
Q60 Overeating	0.25 <0.02	0.26 <0.02	0.23 <0.03
Q63 Having urges to beat injury or harm somebody	-	0.24 <0.03	0.22 <0.04
Q122 Earaches	0.26 <0.05	-	-
Q26 Blaming yourself for things	0.26 <0.02	-	-
Q80 Feeling that something bad is going to happen to you	0.25 <0.02	-	-
Q5 Loss of sexual interest or pleasure	0.24 <0.03	-	-
Q33 Feeling fearful	0.22 <0.04	-	-
Q6 Feeling critical of others	0.21 <0.05	-	-
Q64 Awakening early in the morning	-	0.25 <0.02	-
Q89 Feelings of guilt	-	0.24 <0.03	-
Q123 Facial pain	-	0.23 <0.04	-
Q67 Having urges to smash or break things	-	-	0.26 <0.01
Q10 Worried about sloppiness or carelessness	-	-	0.22 <0.05
Q24 Temper outbursts that you cannot control	-	-	0.22 <0.05
Control Subjects			
Q103 Sore glands	0.31 <0.008	0.33 <0.005	-
Q93 Constipation	0.29 <0.02	0.29 <0.02	-
Q124 Swallowing difficulties	0.28 <0.02	-	-
Q53 A lump in your throat	0.26 <0.03	-	-
Q57 Feeling tense or keyed up	0.24 <0.04	-	-
Q13 Feeling afraid of open spaces	-	0.40 <0.0005	-
7-day Severity regression model; R^2=0.955, F(16,38)=50.212, *P*<0.00000 Variables: 1) Frightening thoughts or images *P*<0.000001, 2) Trouble falling asleep *P*<0.000001, 3) Idea being punished for sins -*P*<0.000001			
12-Month Frequency regression model; R^2=0.947, F=24.980, *P*<0.00000 Variables: 1) Constipation *P*<0.00002, 2) Difficulty making decisions -*P*<0.000001, 3) Avoiding certain things or places because they frighten you *P*<0.000001			
12-Month Severity regression model; R^2=0.934, F=18.527, *P*<0.00000 Variables: 1) Feeling low in energy -*P*<0.00002, 2) Lump in throat *P*<0.02, 3) Thoughts of ending your life *P*<0.002			

In the control group DDE was positive correlated with urine volume, serum ethanolamine, the urinary tyrosine: leucine ratio and the total excreted organic acids, the percentage monocytes and the urinary hippuric acid and citric acid levels. In the control subjects DDE was negatively correlated with the urinary relative abundance of phenylalanine, hydroxyproline, tyrosine, threonine, leucine and UM17, the serum tyrosine: leucine ratio and the total serum amino acid levels. Table 3.37 shows the multi- and uni-variant analyses of the associations between Deildrin and the 7-day, 12-month severity and frequency of symptoms. Deildrin was associated with the 12-month frequency of facial pain in the CFS patients but not in the control subjects. Thus DDE and Deildrin are both associated with the long-term frequency and severity of TMD and not the 7-day pain process.

Table 3.38 shows the multi- and uni-variant analyses of the association between Deildrin and the metabolic parameters in the CFS and control groups. In the CFS group the primary multiple regression variable for increasing Deildrin levels was serum s-methylcysteine whilst in the control group the primary variable was the unknown, serum Ph2a which is the primary serum variable which determines the difference between CFS patients and control subjects. The uni-variant analyses showed that increasing Deildrin levels

Table 3.38. Summary of the multiple regression and correlation analysis associations between Deildrin and biochemistry in the CFS patients and control subjects.

Parameter	CFS	Control
Age	0.23 <0.03	0.06
Duration	0.01	-
Positive Correlations		
Serum S-Methylcysteine	0.60 <0.001	0.15
% Urine Ornithine	0.36 <0.001	0.15
Total serum organic acids	0.34 <0.001	0.05
% Urine Ethanolamine	0.34 <0.001	-0.01
% Urine Acetaminophen	0.27 <0.007	-0.06
% Urine UM15a	0.26 <0.02	-0.08
Serum Aspartic acid	0.24 <0.02	-0.13
% Urine Tyrosine	0.23 <0.03	-0.01
% Urine UM15	0.22 <0.04	0.01
Serum Ph2a	0.02	0.26 <0.02
MCHC	0.11	0.25 <0.03
% Urine Aconitic acid	-0.01	0.23 <0.05
Negative Correlations		
% urine UM14	-0.26 <0.02	0.02
EPM urine UM28	-0.25 <0.02	0.09
Serum Alanine	-0.24 <0.02	-0.11
CFS regression model; R^2=0.870, F=4.476, P<0.0009		
Variables; 1) Serum S-methylcysteine P<0.0005, 2) %U Ornithine P<0.002, 3) %U 1-methylhistidine P<0.004		
Control regression model; R^2=0.608, F=3.225, P<0.0002		
Variables; 1) Serum Ph2a P<0.000005, 2) % U lysine P<0.05, 3) %U Tyrosine P<0.008		

in the CFS group were associated with increases in serum s-methylcysteine, aspartic acid and the total serum organic acids and the urinary relative abundance of ornithine, ethanolamine, acetaminophen, UM15a and UM15. In the control group Deildrin was positively correlated with serum Ph2a, the urinary relative abundance of aconitic acid and the mean erythrocyte haemoglobin concentration. In the CFS group Deildrin were negatively correlated with serum alanine, the urinary relative abundance of UM14 and the excretion rate per minute of UM28. Thus increasing DDE and Deildrin levels were was associated with many biochemical parameters that are associated with 12-month frequency and severity of TMD symptoms.

3.4.2.x. Association between Antibiotic Use, Symptoms and Biochemical Parameters.

In Chapter 2 CFS patients with facial pain were found to have an increased prevalence of antibiotic use at onset, repeated prescriptions of antibiotics and having taken antibiotics for >4 weeks duration. Table 3.39 summarises the biochemical parameters found to differ between the CFS patients who reported multiple antibiotic use or prolonged antibiotic use (>3 months) and the remaining CFS patients.

Table 3.39. Summary of the multiple regression and univariate analyses of the association between the biochemical parameters and 1) prolonged antibiotic use; and 2) prolonged stress; in CFS patients.

Parameter t-Test	**Antibiotic Use**	**No Antibiotics**	***P*-Value**
Multiple Antibiotics	Mean (Std.Err.)	Mean (Std.Err.)	
Increased			
DDE	4.46(1.04)	2.48(0.41)	<0.05
Total Urinary organic acids excreted/minute	3871(525)	2772(441)	<0.05
Urinary EPM UM15a	33.1(9.8)	18.9(3.4)	<0.05
Decreased			
Serum aspartic acid	1372(128)	1912(277)	<0.007
Serum serine	1469(131)	1769(184)	<0.02
% U proline	1.61(0.13)	2.17(0.23)	<0.03
%U β-alanine	0.81(0.09)	1.14(0.14)	<0.03
Prolonged Antibiotics (>4 weeks)			
Increased			
DDE	4.96(1.15)	2.34(0.32)	<0.02
% Basophils	0.40(0.04)	0.26(0.04)	<0.02
% Lymphocytes	34.1(1.15)	30.4(1.36)	<0.04
Serum Tyrosine: leucine	0.35()	0.29()	<0.04
Decreased			
Serum Isoleucine	1540(86.8)	1426(96.1)	<0.02
Neutrophil: lymphocyte ratio	1.74(0.10)	2.15(0.15)	<0.03
Multiple Antibiotics Forward stepwise regression model: R^2=0.754, F=3.825, *P*<0.001 Serum aspartic acid -*P*<0.003; 2) Serum serine -*P*<0.0005; 3) Serum leucine -*P*<0.05 **Prolonged Antibiotics** Forward stepwise regression model: R^2=0.721, F=3.224, *P*<0.004 DDE *P*<0.49; 2) Serum valine -*P*<0.00004; 3) Serum PH2 *P*<0.0003			
Prolonged Stress	**Stress**	**No Stress**	***P*-Value**
Increased			
Bilirubin	11.9(1.32)	8.8(0.49)	<0.03
Serum glycine	3741(247)	3363(257)	<0.05
Decreased			
Serum protein	71.7(0.65)	73.5(0.59)	<0.04
Prolonged Stress Forward stepwise regression model: R^2=0.716, F=3.149, *P*<0.004 1) Bilirubin *P*<0.00005; 2) Serum protein -*P*<0.03; 3) ALT *P*<0.09			

Multiple regression analysis revealed that the fall in serum aspartic acid levels was the primary variable associated with multiple antibiotic use whilst an increase in DDE was the primary variable associated with prolonged antibiotic use. CFS patients who reported multiple antibiotic use had increases in DDE, urinary relative abundance of UM15a and the total urinary organic acids excreted and reductions in serum aspartic acid and serum and urinary relative abundance of proline and β-alanine. CFS patients taking prolonged courses of antibiotics had increases in DDE, percentage basophils and lymphocytes and an increase in the serum tyrosine: leucine ratio, as well as reductions in serum isoleucine and the neutrophil: lymphocyte ratio. Thus prolonged use of antibiotics was associated with increases in the percentage basophils and DDE and a reduction in the neutrophil: lymphocyte ratio.

3.4.2.y. Association between Prolonged Stress, Symptoms and Biochemical Parameters.

In chapter 2 prolonged stress over the previous 5-8 years was higher in CFS patients with TMD symptoms, compared with CFS patients without TMD symptoms therefore analysis of the biochemical changes associated with prolonged stress were assessed. Table 3.39 summarises the multi- and uni-variant analyses and shows that an increase in serum bilirubin levels was associated with the reporting of prolonged stress. CFS patients reporting prolonged stress were found to have increased bilirubin and serum glycine levels and reduced serum protein levels. These data show that increased reporting of stress is associated with alteration in markers of liver dysfunction.

3.4.2.z. SCL-90-R Somatization and Depression Dimensions and Biochemical Parameters.

Axis II of the RDC/TMD criteria requires the investigation of the SCL-90-R somatization and depression dimension scores. Multi- and uni-variant analyses are shown in Table 3.40 and there show that the primary variable for the somatization dimension was urine volume whilst for the depression dimension the urinary relative abundance of proline was the primary variable. Correlation analysis showed that the somatization scores were associated with increases in urine volume, ESR, leukocyte count and serum Ph1, and reductions in serum sodium and alanine levels as well as the erythrocyte haemoglobin levels. The depression dimension scores were associated with increases in serum potassium, mean platelet volume and the eosinophil count, and reductions in the urinary relative abundance of proline, hydroxyproline and threonine, mean erythrocyte haemoglobin concentration, and the two pesticides, Heptochlor and Aldrin. Thus the somatization dimension is strongly associated with biochemical changes associated with TMD expression whilst the changes associated with the depression dimension scores were quite different and unrelated to those seen with TMD symptom expression.

Table 3.40. Summary of the multiple regression and univariate analyses of the association between the biochemical parameters and the SCL-90-R somatization and depression dimensions in CFS patients.

Parameter	Somatization dimension
Correlation	
Increased	
Urine volume	0.32 <0.002
ESR	0.24 <0.05
Total leukocyte count	0.22 <0.04
Serum Phl	0.21 <0.04
Decreased	
Serum Sodium	-0.23 <0.03
Serum Alanine	-0.23 <0.03
Mean erythrocyte haemoglobin	-0.22 <0.03
Somatization Forward stepwise regression model: R^2=0.741, F=3.572, P<0.002 1) Urine volume P<0.05; 2) ESR P<0.0001; 3) Serum Proline -P<0.01	
Correlation	Depression dimension
Increased	
Serum potassium	0.24 <0.03
Mean Platelet Volume	0.23 <0.02
Eosinophil count	0.23 <0.03
Decreased	
% urinary Proline	-0.31 <0.003
% urinary hydroxyproline	-0.29 <0.006
MCHC	-0.29 <0.01
Pesticide Heptochlor	-0.22 <0.04
% urinary threonine	-0.21 <0.05
Pesticide Aldrin	-0.21 <0.05
Depression Forward stepwise regression model: R^2=0.580, F=2.768, P<0.009 1) % urinary Proline -P<0.001; 2) MCHC -P<0.23; 3) Eosinophil count P<0.03	

3.4.2.aa. Sex differences in Symptoms and Biochemical Parameters.

Multiple regression and Spearman rank correlation analyses were applied to determine whether there were differences between males and females in their responses to TMD symptoms. Table 3.41 shows the multiple regression analyses whilst 3.41 shows the Spearman rank correlation analyses of the different TMD responses in the CFS patients. The 7-day severity of facial pain in the female CFS patients was associated with a strong regression model with a reduction in serum sodium levels being the primary discriminant variable. In the male CFS patients the regression model was also strong, however the primary discriminant variable was a reduction in serum lysine. The 7-day severity of TMJ pain in the female CFS patients was associated with a strong regression model with an increase in serum alanine levels being the primary discriminant variable. In the male CFS patients the regression model was also strong with the organochlorine pesticide, Deildrin, being positively correlated and the only regression variable.

Table 3.41. Summary of the forward stepwise regression analyses of the differences in the various TMD symptoms between females and males in the CFS patient group.

Parameter	Female Model/Variables	Male Model/Variables
Facial Pain		
7-day Severity	R^2=0.994, F=176.92, P<0.00000 Variables 1) Sodium, -<1.0E^{-13} 2) Creatinine, +<6.0E^{-10} 3) Calcium, +<2.0E^{-10}	R^2=0.770, F=8.918, P<0.007 Variables 1) Serum Lysine -<0.004 2) Serum aminohydroxyproprionate -<0.04 3) Neutrophil count +<0.05
12-month Severity	R^2=0.988, F=54.067, P<0.00000 Variables 1) ALT +<0.002 2) Protein +<0.0003 3) Iron +<0.000002	R^2=0.862, F=18.691, P<0.00004 Variables 1) Haemoglobin +<0.00001 2) Red cell distribution width +<0.002 3) ANA Titre -<0.0007
12-month Frequency	R^2=0.981, F=24.931, P<0.00001 Variables 1) Protein +<0.05 2) Basophil count +<0.33 3) Serum Proline -<0.001	R^2=0.758, F=13.548, P<0.0003 Variables 1) Haemoglobin +<0.0002 2) Red cell distribution width +<0.002 3) Haematocrit -<0.003
TMJ Pain		
7-day Severity	R^2=0.964, F=38.893, P<0.00000 Variables 1) Serum alanine +<0.00000008 2) Serum Vit B_{12} -<0.00000003 3) Serum serine +<0.0000000004	R^2=0.681, F=21.305, P<0.001 Variables 1) Deildrin +<0.001
12-month Severity	R^2=0.998, F=357.73, P<0.00000 Variables 1) Serum glycine -<1.5E^{-11} 2) Serum β-aminisobutyrate +<1.7E^{-12} 3) ESR +<1.0E^{-14}	R^2=0.953, F=44.072, P<0.00000 Variable 1) Excret. Rate/minute glycine +<1.0E^{-6} 2) Vit B12 +<0.0003 3) Serum alanine -<0.000007
12-month Frequency	R^2=0.985, F=14.161, P<0.004 Variables 1) Protein +<0.002 2) Acetaminophen +<0.19 Serum Phl +<0.22	R^2=0.916, F=32.925, P<0.00000 Variables 1) Iron -<0.0000005 2) Haematocrit +<0.00002 3) Bilirubin +<0.0003

Table 3.42 shows that the 7-day severity of facial pain and TMJ pain were unrelated to age, duration or symptom prevalence in either males or females. In the females both facial pain and TMJ pain were associated with an increase in urine volume whilst facial pain was additionally associated with a reduction in serum sodium. In males the 7-day facial and TMJ pain severity were associated with increases in Deildrin, monocytes, the neutrophil: lymphocyte ratio, red cell distribution width and iron levels. These data show that 7-day facial and TMJ pain severity in male and female CFS patients represent different biochemical

processes. Females have evidence of hyponatraemia, whilst males have evidence of Deildrin accumulation and a neutrophil-mediated pain response.

The 12-month frequency and severity of facial pain in the female CFS patients were associated with strong regression models with an increase in ALT and serum protein, respectively, being the primary discriminant variables. The 12-month frequency and severity of facial pain in the male CFS patients were associated with strong regression models with an increase in haemoglobin being the primary discriminant variable in both analyses. The 12-month frequency and severity of TMJ pain in the female CFS patients were associated with strong regression models with an increase in serum protein and a reduction in serum glycine, respectively, being the primary discriminant variables. The 12-month frequency and severity of TMJ pain in the male CFS patients were associated with strong regression models with an increase in the urinary excretion rate of glycine and a reduction in serum iron, respectively, being the primary discriminant variables.

The 12-month facial pain severity in females was associated with increases in urine volume, the basophil count, iron and the serum tissue damage markers, ALT, AST and urea. In males the 12-month facial pain severity was associated with increases in the leukocyte count (neutrophils and basophil) and the serum tissue damage marker ALT. The 12-month facial pain frequency in females was associated with increases in urine volume, the basophil count, iron, protein and the serum tissue damage marker, ALT. In males the 12-month facial pain frequency was associated with increases in the neutrophil count, basophil count, the neutrophil: lymphocyte ratio, serum glucose and potassium levels. The one feature common to both females and males in relationship to the 12-month severity and frequency of facial pain was the positive association with the basophil count.

The 12-month TMJ pain severity in females was not associated with any changes whilst the 12-month TMJ pain frequency was associated with increases in the serum iron levels. In males the 12-month TMJ pain severity was associated with increases in the haematocrit and the total metabolites excreted per minute, and a reduction in serum iron. The 12-month TMJ pain frequency in males was associated with increases in the monocyte count, the total metabolite (both amino and organic acids) and reductions in iron and bilirubin levels.

The cellular and biochemical variables associated with TMD symptoms in females appear to be distinct from the cellular and biochemical variables in males. These data also show variations in the white blood cell parameters and urinary metabolite output between males and females.

Table 3.42. Analysis of the differences in Spearman rank correlation analyses for the various TMD symptoms between females and males in the CFS patient group.

Parameter	Female			Male		
	7-day Severity	12-month Severity	12-month Frequency	7-day Severity	12-month Severity	12-month Frequency
Facial Pain	r P	r P	r P	r P	r P	r P
Age	-	0.27 <0.03	-	-	0.26 NS	-
Duration	-	0.30 <0.02	0.25 <0.05	-	0.30 NS	0.29 NS
Symptom prevalence	-	0.30 <0.02	0.30 <0.02	-	0.78 <0.00002	0.71 <0.0003
White cell count	-	0.12 NS	-	-	0.46 <0.03	-
Neutrophil:Lymphocyte	-	-	0.09 NS	-	-	0.53 <0.01
Neutrophil count	-	0.12 NS	0.14 NS	-	0.52 <0.01	0.48 <0.03
Lymphocyte count	-	-	-0.12 NS	-	-	-0.50 <0.02
Basophil count	-	0.31 <0.01	0.26 <0.04	-	0.45 <0.04	0.48 <0.03
Sodium	-0.36 <0.02	-	-	0.11 NS	-	-
ALT	-	0.36 <0.005	0.27 <0.04	-	0.55 <0.009	0.40 NS
AST	-	0.34 <0.007	-	-	0.30 NS	-
Iron	-	0.31 <0.02	0.30 <0.02	-	-0.35 NS	-0.23 NS
Urea	-	0.30 <0.02	-	-	0.09 NS	-
Protein	-	0.26 <0.05	0.28 <0.03	-	-0.04 NS	-0.34 NS
Potassium	-	-	0.20 NS	-	-	0.44 <0.05
Glucose	-	-	0.09 NS	-	-	0.44 <0.05
Urine volume	-	0.32 <0.01	0.30 <0.02	-	0.06 NS	0.10 NS
TMJ Pain						
Age	-	0.06 NS	0.11 NS	-	0.49 <0.03	0.48 <0.03
Symptom prevalence	-	0.33 <0.008	0.32 <0.009	-	0.39 NS	0.36 NS
Neutrophil:Lymphocyte	0.12 NS	-	-	0.48 <0.05	-	-
Monocyte count	-0.26 NS	-	0.05 NS	0.49 <0.05	-	0.48 <0.03
Red cell distribution width	-0.15 NS	-	-	0.55 <0.02	-	-
Haematocrit	-	0.09 NS	-	-	0.47 <0.03	-
Deildrin	-	-	-	0.64 <0.008	-	-
Iron	-0.19 NS	0.24 NS	0.30 <0.02	0.61 <0.01	-0.72 <0.0002	-0.56 <0.01
Bilirubin	-	-	0.08 NS	-	-	-0.45 <0.05
Urine volume	0.33 <0.03	-	-	0.05 NS	-	-
Metabolite/minute	-	0.04 NS	0.06 NS	-	0.44 <0.05	0.63 <0.003
Amino acids/minute	-	-	0 NS	-	-	0.56 <0.02
Organic acids/minute	-	-	-0.01 NS	-	-	0.50 <0.03

However, increases in the basophil count and the tissue damage marker, ALT, were prominent features of 12-month facial pain severity and frequency in both males and females. The reduction in sodium levels was a predominant feature of TMD expression in females whilst increases in neutrophil numbers with TMD were more prominent in males.

3.4.3. RNase-L Study.

3.4.3.a. Group Differences.

Table 3.43 shows that there was no difference in age or sex between the CFS and control subjects. The CFS patients had an increased prevalence of patients with elevated RNase-L activity, soluble IL-2 receptor (sIL-2r) levels (>2sd over control) and patients with detectable IL-6, as well as increased levels of sIL-2r and IL-6.

Table 3.43. Summary of the discriminant function and univariate analysis differences in the prevalence and concentrations of RNase-L, sIL-2r and IL-6 between the CFS patients and control subjects.

Parameter	CFS	Control	P-Value
Patient Characteristics			
Number	31	31	
Age	46.1±13(X±SD)	40.0±13(X±SD)	NS
% Female	71.9%	83.9%	NS
Prevalence	N (%)	N (%)	
Increased RNase-L activity	23 (71.9%)	4 (12.9%)	<0.0001
sIL-2r (>2SD)	13 (40.6%)	2 (6.5%)	<0.01
Presence of IL-6	18 (56.3%)	3 (9.7%)	<0.0001
Concentration	X(SD)	X(SD)	
sIL-2r	1465±999	711±292	<0.0002
Il-6	70.94±108.33	8.58±28.52	<0.0001
TMD symptoms			
7-Day face pain			
Prevalence	14 (43.8%)	2 (9.7%)	<0.003
Severity score	0.88 (1.2)	0.16(0.6)	<0.003
TMJ lock/click			
Prevalence	10 (31.3%)	6 (19.4%)	NS
Severity score	0.59 (1.1)	0.39(1.0)	NS
Correlation Analysis	**CFS** r *P*	**Control** r *P*	**All subjects** r *P*
7-Day Face pain			
RNase-L	-0.10 NS	-0.03 NS	0.14 NS
sIL-2r	0.10 NS	-0.02 NS	0.24 NS
IL-6	-0.10 NS	-0.11 NS	0.10 NS
7-Day TMJ locking/clicking			
RNase-L	0.07 NS	-0.03 NS	0.09 NS
sIL-2r	-0.07 NS	-0.23 NS	-0.04 NS
IL-6	0.05 NS	-0.16 NS	0.05 NS
Group Forward stepwise discriminant function analysis; Wilks' λ=0.643, F(3,58)=10.736, *P*<0.0000 Variables 1) RNase-L *P*<0.03; 2) sIL-2r *P*<0.004; 3) IL-6 *P*<0.02			
Face pain Forward stepwise regression analysis; R^2=0.046, F=2.876, *P*<0.10 (Model not significant)			
TMJ locking/clicking No model – No associations.			

Multiple regression analysis revealed a strong difference between the CFS patients and the control subjects ($P<0.00000$) with the principle difference being an increase in RNase-L ($P<0.03$), followed by sIL-2r ($P<0.004$), and IL-6 ($P<0.03$). Thus CFS patients have increased RNase-L, sIL-2r and IL-6 levels with the primary discriminant variable being the increase in RNase-L activity. Spearman rank order correlation analysis found a positive association between RNase-L and both IL-6 (r=0.55, $P<0.000003$) and sIL-2r (r=0.29, $P<0.03$), but no association between sIL-2r and IL-6 levels (r=0.14) in the entire study population. No association was found between RNase-L, sIL-2r or IL-6 in the CFS group. CFS patients had an increased odds ratio (OR=18.0 χ^2=19.19, $P<0.00001$) for an elevated RNase-L test.

3.4.3.b. TMD Symptom Associations.

The CFS patients had an increased prevalence and scalar response for 7-day facial pain compared with the control subjects (Table 3.43). TMJ locking/clicking was not increased in CFS patients compared with controls. Neither multiple regression nor univariate analysis found any association between 7-day facial pain or 7-day TMJ locking/clicking and RNase-L, sIL-2r or IL-6. No sex differences were found in any parameter in relationship to TMD symptoms. The correlation analyses between symptom expression and RNase-L, sIL-2r or IL-6 are shown in Tables 3.44a and 3.44b, respectively. These data show that variation in each of the immune activation markers and RNase-L were associated with different symptom patterns. Thus TMD symptoms were not associated with alterations in RNase-L or the two immune activation markers, sIL-2r or IL-6. However symptom expression was altered by the different measures assessed.

Table 3.44a. Summary of the Spearman correlation analysis associations between symptoms and RNase-L in all subjects, the CFS patients and control subjects.

Assessment	All Subjects		CFS		Control	
RNase-L	r	*P*	r	*P*	r	*P*
Headaches	0.38	<0.002	-0.01	-	-0.06	-
Neck pain	0.27	<0.04	0.14	-	0.07	-
Shoulder pain	0.28	<0.03	0.12	-	-0.02	-
Arm pain	0.44	<0.0004	0.17	-	0.19	-
Abdominal pain	0.26	<0.04	0.11	-	0.28	-
Leg pain	0.44	<0.0003	0.22	-	-0.12	-
Myalgia	0.42	<0.0006	0.11	-	-0.02	-
Muscle weakness	0.46	<0.0002	0.19	-	-0.22	-
Paraesthesia	0.49	<0.00005	0.43	<0.02	-0.01	-
Fever	0.38	<0.002	0.18	-	0	-

Table 3.44b. Summary of the Spearman correlation analysis associations between symptoms and sIL-2r and IL-6 in all subjects, the CFS patients and control subjects.

Assessment	All Subjects		CFS		Control	
Soluble IL-2 receptor	r	P	r	P	r	P
Headaches	0.34	<0.007	0.01	-	0.22	-
Neck pain	0.42	<0.0007	0.28	-	0.10	-
Shoulder pain	0.43	<0.0004	0.40	<0.03	0.07	-
Abdominal pain	0.32	<0.02	0.34	-	-0.18	-
Low back pain	0.45	<0.0003	0.19	-	0.45	<0.01
Leg pain	0.43	<0.0006	0.36	<0.05	-0.02	-
Sciatica	0.53	<0.00001	0.53	<0.002	0.37	<0.05
Arthritis	0.49	<0.00006	0.40	<0.03	0.17	-
Myalgia	0.44	<0.0004	0.19	-	0.31	-
Muscle weakness	0.40	<0.002	0.14	-	0.14	-
Paraesthesia	0.29	<0.03	0.22	-	-0.28	-
Fever	0.35	<0.006	0.23	-	0	-
IL-6						
Arm pain	0.31	<0.02	0.08	-	-0.13	-
Low back pain	0.25	<0.05	0.02	-	-0.22	-
Myalgia	0.36	<0.004	-0.07	-	-0.16	-
Muscle weakness	0.33	<0.009	-0.08	-	-0.14	-
Paraesthesia	0.29	<0.03	0.13	-	-0.11	-

3.5. Discussion.

This chapter considered the association between TMD symptoms and alterations in biochemical and blood cell parameters. The data were assessed as group differences (RDC/TMD type 1a pain verses controls; CFS patients with TMD symptoms verses CFS patients without TMD symptoms) and the associations between the TMD symptom expression and alterations in various biochemical and blood cell parameters (prevalence, 7-day severity, 12-month severity, 12-month frequency). These data also assessed the variation in TMD expression in CFS patients and controls and indicated that the biochemical associations with TMD symptoms were homogeneous and independent from the alterations that defined CFS patients from controls. The homogeneous nature of TMD symptom and biochemical parameters suggests that TMD symptom expression is associated with a distinct set of biochemical changes that may be initiated by different stimuli. TMD symptoms were associated with alterations in urinary excretion of markers of proteolysis and protein synthesis. The excitatory amino acids were seen to be similar in the RDC/TMD type 1a muscle pain group and the CFS patients with TMD symptoms. Similarly, increases in the neutrophil count associated with TMD symptom expression was seen in both CFS and control groups in the CFS study. This is consistent with the symptom associations reported in Chapter 2 and the study by Plesh et al (1996). Plesh et al (1996) assessed both TMD and fibromyalgia patients and found that 18% of TMD patients suffered from fibromyalgia whilst 75% of fibromyalgia patients had TMD symptoms. They concluded that TMD was a distinct disorder that occurred in increased prevalence in fibromyalgia patients. In this study, 69% of the CFS patient group had TMD symptoms, whilst 20% of the CFS control group had TMD symptoms, which is consistent with the observations by Plesh et al (1996). From these data it may be concluded that TMD symptom expression is associated with:

1) a distinct set of biochemical processes;
2) dysregulated urinary excretion of markers of proteolysis, protein synthesis;
3) increased excretion of excitatory amino acids, aspartic and glutamic acid; and
4) evidence of immune dysregulation, neutrophil and basophil counts.

These data suggest that in CFS patients, TMD symptoms may be either a distinct host-based response or the result of a distinct group of non-host agents or factors that induce the same host response. CFS and fibromyalgia patients have an increased prevalence of TMD symptoms (Plesh et al, 1996, McGregor et al, 1996), which suggests that CFS and fibromyalgia patients may have a co-factor associated disease condition or a secondary opportunistic infection that aggravates patient morbidity. This state of coexisting disease may also exist for CFS and fibromyalgia. Bombardier & Buchwald (1996) showed that of 402 patients who attended a fatigue clinic that CFS and fibromyalgia were diagnosed in 52% and 22%, respectively. Thirty-seven percent were diagnosed with CFS alone, 7% with fibromyalgia alone whilst 15% were diagnosed to have both CFS and fibromyalgia. The patients with both CFS and fibromyalgia had greater disability than patients diagnosed to have either condition alone (Bombardier & Buchwald, 1996; Buchwald et al, 1996). Thus this study indicates that TMD is a distinct clinical and biochemical condition which occurs in increased incidence in CFS patients and is associated with increased morbidity.

The RDC/TMD type 1a group had a different excretion of amino acids compared with the control group. The increase in urinary amino acid output in the RDC/TMD type 1a group that was associated with increasing pain severity was also seen in the CFS patients in relation to their musculoskeletal pain symptoms. The RDC/TMD type 1a pain group had a reduced excretion of leucine, which is an important regulator of non-fibrillar proteolysis (Mortimore & Poso, 1993) and muscle fatigue (Wagenmaker, 1998). The relative abundance of serine, alanine, glycine, valine, threonine, and glutamic acid, which also modulate proteolysis, had reduced excretion in the RDC/TMD type 1a pain group. The urinary tyrosine: leucine ratio, which is a suggested indicator of the balance between proteolysis and protein synthesis, was increased in the RDC\TMD type 1a pain group and was positively correlated with pain severity, muscle pain scores, symptom prevalence and increases in body pain distribution. Unlike the RDC\TMD type 1a pain group CFS patients with TMD symptoms appeared to have an increase in tyrosine excretion, without a significant change in leucine, yet the tyrosine: leucine ratio was still a good indicator of TMD symptoms. In the CFS patients with TMD symptoms, the increase in tyrosine excretion and the tyrosine: leucine ratio, without the reduction in leucine excretion, suggests that variation in proteolysis and not protein synthesis was more important in TMD symptom expression in the CFS patients. Therefore the urinary tyrosine: leucine ratio was a better indicator of TMD symptoms than either tyrosine or leucine alone when both studies were assessed. **It is proposed that a tyrosine: leucine ratio of >4.2 may therefore be used as a diagnostic predictor of chronic RDC/TMD type 1a pain.** These data provide reproducible evidence of an association between TMD symptoms and dysregulated amino acid metabolism indicative of increased proteolysis and reduced protein synthesis.

The urinary tyrosine: leucine ratio was positively associated with symptom prevalence, pain severity, increasing body pain distribution, muscle pain scores, SCL-90-R somatization scores and urine volume but not illness duration, SCL-90-R depression scores, a sudden onset of illness or fatigue. The serum tyrosine: leucine ratio was quite different from the urinary tyrosine: leucine ratio and was inversely related. Increases in the urinary tyrosine levels were associated with reductions in the serum tyrosine concentrations. This anomaly was not seen with any other amino or organic acid, suggesting that some alteration in renal handling of tyrosine may be occurring in relation to the chronic pain response.

In the RDC/TMD type 1a pain patients, the total metabolite excreted was positively associated with increases in pain severity and pain distribution and negatively correlated with the duration of the illness. The total metabolite excreted was also higher in those RDC/TMD type 1a pain patients where there was a sudden onset. In chapter 2 as in other studies (Weinberg & Lager, 1980; Fricton et al, 1985; Bengtsson et al, 1986) 60% of chronic RDC/TMD type 1a pain patients reported a gradual onset of symptoms and most a localised pain pattern at onset which progressed to a more wide-spread pain distribution. These data suggest that at onset, there is an increase in urinary metabolite loss that repetitively increases with each period of pain exacerbation, and results in a net progressive loss of metabolites as displayed hypothetically in Figure 3.13. In CFS patients, neither the total serum nor urinary amino acids levels were different, however the ratio of serum amino acid: urinary excretion rate per minute of amino acids was increased. Similarly increases in urine volume and the metabolites excreted also positively correlated with facial pain severity yet the volume of urine excreted was less in the CFS group compared with the control group. These data are consistent with the aminoaciduria that occurs with trauma associated pain (Jeevanandam, 1995) which results in loss of metabolite and may result in the reduction in total protein and RNA in painful muscle (Young et al, 1970; Pacy et al, 1988). These data therefore provide the potential biochemical basis for a gradual pain onset, and the progression of symptom severity and pain distribution, where the progressive loss of metabolites is associated with a gradual increase in pain distribution and severity.

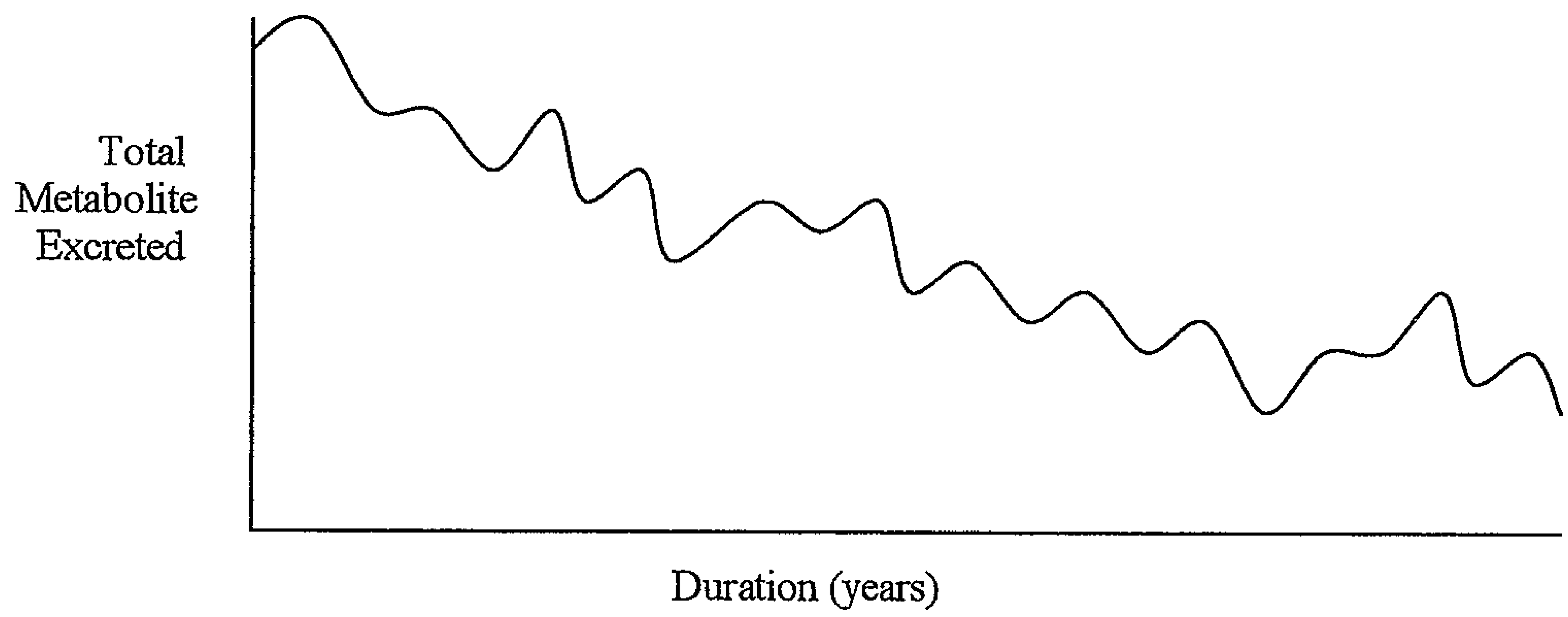


Figure 3.13. Aminoaciduria, Metabolite Depletion and Pain – the proposed scheme for development of a gradual onset of chronic RDC/TMD type 1a pain. Repetitive bouts of amino acid release from body stores (muscle, liver) result from an increase in catabolic processes. The excess circulating amino acids are lost from the kidney through a nitric oxide mediated event. This prolonged repetitive process results in depletion of body amino acid stores and the development of more widespread pain and increased dysregulation of multi-organ homeostasis.

In the sudden onset RDC/TMD type 1a patients the total urinary metabolite was higher suggesting that the factors associated with the sudden onset resulted in an exacerbated response. This enhanced or exacerbated response would result in a more rapid rate of metabolite loss and a more sudden onset of chronic pain symptoms and therefore complies with the model shown in figure 3.13.

Table 3.45 shows the biochemical parameters that altered with illness duration and pain severity in the RDC/TMD type 1a pain patients and the biochemical mechanisms in which they are involved. Average pain severity in the RDC/TMD type 1a patients, as assessed by a VAS, was positively correlated with the total urinary amino acids excreted, the tyrosine: leucine ratio, the proteolysis marker, tyrosine, the excitatory amino acids, aspartic acid and glutamic acid, and the phospholipid polar head-group amino acids, serine and ethanolamine. The increased excretion of total metabolites and excitatory amino acids was also seen in the CFS group with TMD symptoms. There was a significant difference between the total amino and organic acids excreted and the relative abundance of the excreted amino and organic acids in both the RDC/TMD type 1a patients and the CFS patients with TMD symptoms. Increasing 12-monthly facial pain

Table 3.45. Summary of the potential metabolic associations which may be effected by the various changes in urine excretion which were correlated with illness duration and pain severity.

Urine excretion anomaly	Interpretation/associated metabolism
Pain severity was associated with:	
Reductions in the excretion of:	
leucine	Indicator of protein synthesis, regulator of protein catabolism
Increases in the excretion of:	
total metabolites	Kidney resorption – nitric oxide diuresis, catabolism
tyrosine: leucine ratio	A measure of protein catabolism to protein synthesis
aspartate, glutamic acid	Excitatory amino acids, nitrogen metabolism, transamination, gluconeogenesis, nitric oxide precursors via AST and argininosuccinate synthetase
ethanolamine	Present in the polar head group of important complex lipids as components of cell membranes
serine	A precursor to glycine and ethanolamine; required for the formation of tetrahydrofolate derivatives; Present in the polar head group of important complex lipids as components of cell membranes
aconitic acid	Citric acid cycle intermediate
glycine	Neurotransmitter; used in liver as a conjugate to form hippuric acid; formation of bile salts
proline	A connective tissue amino acid
phenylalanine	An essential amino acid; precursor for catecholamine synthesis
valine	An essential branched chain amino acid
UM15a and UM17	Unknown urinary metabolites
Illness duration was associated with:	
Reductions in the excretion of:	
total metabolites	Kidney resorption – nitric oxide diuresis, catabolism
succinic acid	The citric acid cycle and mitochondrial oxidative phosphorylation
hydroxyproline	connective tissue turnover
alanine, glutamic acid/glutamine	Nitrogen metabolism, transamination, gluconeogenesis (see figure 3.2)
hippuric acid	Impaired urea cycle function, liver detoxification
threonine	An essential amino acid
Increases in the excretion of:	
3-methylhistidine	Marker of fibrillar catabolism
UM27	Unidentified urinary metabolite

frequency and severity scores were not associated with a major difference in the urinary relative abundance in the CFS group. These data show that increasing pain severity was associated with an increase in the total amino acids excreted without any significant change in the urinary amino acid concentration. This was also confirmed in the CFS patients where increases in urine volume were positively associated with the pain scores and excitatory amino acid excretion, but not associated with variation in the relative abundance of the majority of metabolites. The only metabolite to increase in relative abundance in the CFS group in relationship to urine volume was lysine, the urea cycle inhibitory amino acid. Lysine was also associated with increases in total amino acid levels and highly correlated with the urea cycle component, ornithine, suggesting that an alteration in nitric oxide levels may be associated with alteration in the lysine level and urine volume.

These data are consistent with an association between increasing pain and increasing total urinary volume without any change in concentration of metabolites. The reduction in urine volume noted in the CFS patients in this study is the same as that noted in fibromyalgia patients (Yunus et al, 1993) and importantly, in the fibromyalgia patients where the urine volume was associated with changes in urinary dopamine levels. These data therefore suggest that dysregulation of nitric oxide and catecholamine metabolism is occurring in chronic pain patients and is consistent with a progressive onset, increasing pain distribution and severity. These data are supported by the observation that increasing pain severity was associated with dysregulation of protein turnover, increases in phospholipid polar head-group amino acid excretion and the citric acid cycle intermediate aconitic acid. Increases in phospholipid turnover are also noted with increases in pain and the release of fatty acids such as arachidonic acid are also involved in development of the pain response (Coderre & Yashpal, 1994). Degradation of citrate to isocitrate with the intermediate aconitic acid occurs by the enzyme aconitase, which is inhibited by nitric oxide (Andersson et al 1998). Thus pain severity in RDC/TMD type 1a patients is associated metabolic changes suggestive of a nitric oxide mediated diuresis, dysregulated protein turnover, increased phospholipid turnover and excitatory amino acid availability, and increased provision of precursors for nitric oxide production. These data are consistent with those proposed by Coderre & Yashpal (1994) for phase 2 of the acute pain response. Figure 3.14 shows a hypothetical acute pain model consistent with the data from this study and Coderre & Yashpal (1994). In a chronic pain model as noted in this study increases in nitric oxide, excitatory amino acids and phospholipid turnover were associated with development of the pain response. This suggests that activation of the spinal hyperalgesic response is occurring without the necessity of the phase 1 peripheral stimulus as noted in the acute pain mechanisms reviewed by Coderre & Yashpal (1994). Thus the hyperalgesic components (phase 2 and 3) of the acute pain model are applicable to the chronic pain model, the major difference being the mode of activation of the response. The chronic pain model would therefore require the presence of a persistent stimulus that activates the spinal hyperalgesic response leading to increases in nitric oxide, excitatory amino acids, phospholipid and protein turnover, and alterations in kidney function.

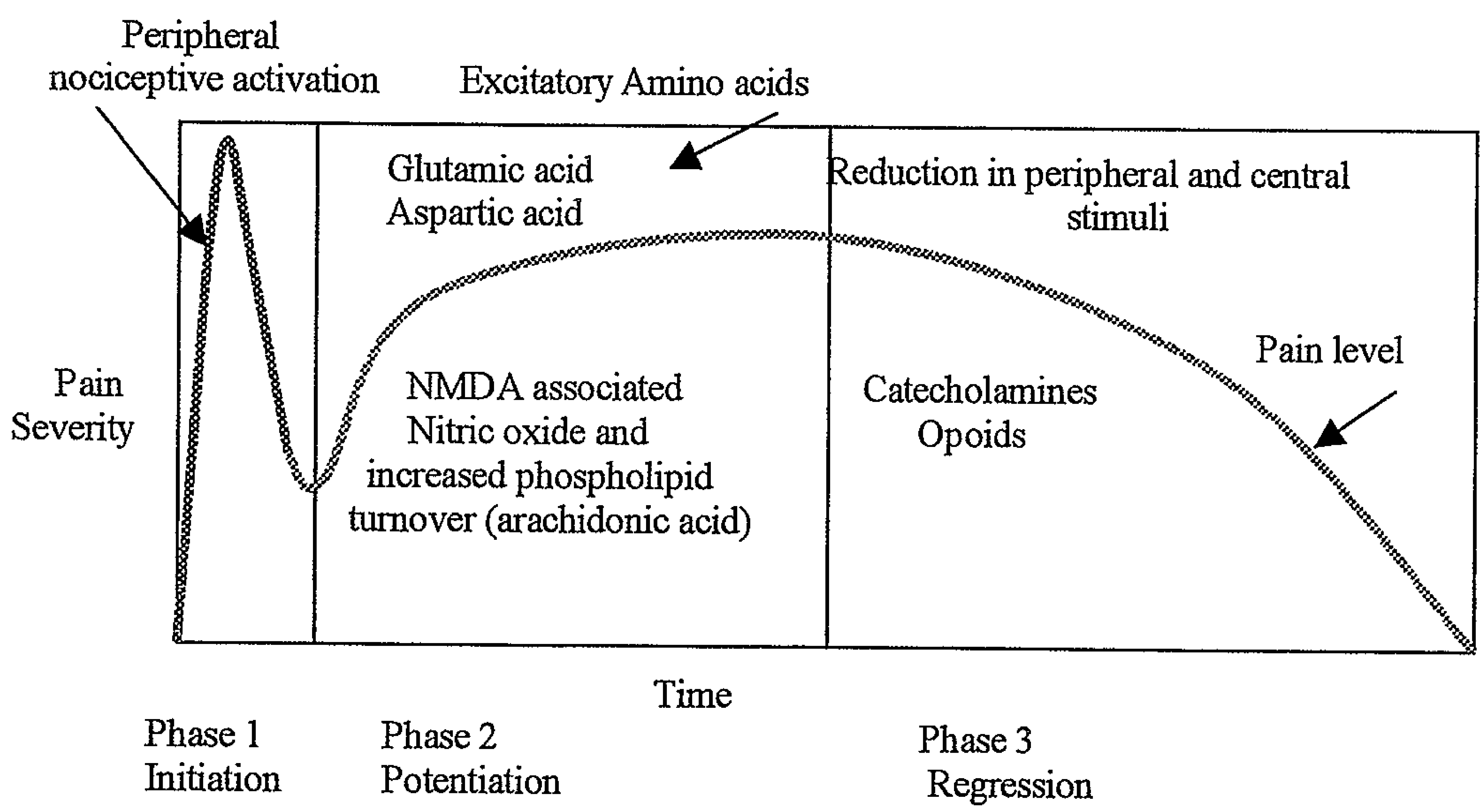


Figure 3.14. Summary of the phases of the hyperalgesia pain response in the spinal cord related to pain severity.
Phase 1. Peripheral nociceptive activation of the pain response. e.g. trauma to tissue.
Phase 2. Potentiation of hyperalgesic response in the spinal column. Increased excitatory amino acid levels activate the NMDA receptor and initiate a nitric oxide mediated response. Na^+K^+ATPase activity is inhibited (Tavalin et al, 1997; Sato et al, 1997) and the hyperalgesic response is initiated.
Phase. 3. A reduction in the peripheral stimuli and potentiation phase components along with increases in catecholamines and opoids inhibit the hyperalgesic response.

Unlike pain severity, in the RDC/TMD type 1a pain group illness duration was associated with reductions in total concentrations of alanine and glutamate/glutamic acid. This strongly suggests a reduction in the ability of muscle and liver to metabolise and mobilise nitrogen, a reaction predominately controlled by the enzyme alanine aminotransferase (ALT). Figure 3.2 shows the basic ALT reaction and how it relates to the normal interchange of glucose and alanine between liver and muscle. If alanine and glutamic acid were substantially depleted in a patient, then this could potentially have a negative impact on the liver's capacity to undergo gluconeogenesis. The depletion of these amino acids may therefore explain in part why some of these patients have episodes of hypoglycaemia (Oles et al, 1978). Illness duration was also associated with an increased relative abundance of 3-methylhistidine, which can be interpreted as evidence for an increase in the degradation of contractile or fibrillar proteins (3-methylhistidine is a unique component of the contractile protein, actin) (Thompson et al, 1996; Rathmacher & Nissen, 1998). This increase in fibrillar proteolysis would be a normal response to a reduction in the ability to provide amino acids from other sources. In keeping with this illness duration was also associated with a reduction in hydroxyproline, which may be interpreted as an indication that connective tissue is also a site for amino acid acquisition during periods of catabolism that may influence TMJ integrity (Axelsson et al, 1992; Axelsson, 1993). This potential dysregulation of connective tissue turnover is also supported by the increase in proline excretion noted with increasing pain severity in this study. Thus illness duration appears associated with a reduction in the ability of the body to provide alanine from muscle, other amino acids from connective and other tissues, dysregulated short-term proteolysis and an increased fibrillar degradation rate. These data are consistent with the reduction in total protein in painful muscle (Young et al, 1970; Pacy et al, 1988; Preedy et al, 1993).

Tyrosine and phenylalanine are the precursor amino acids for the production of catecholamines, dopamine, noradrenaline, and adrenaline. The CFS patients who responded positively to the 7-day severity of facial pain had reduced serum levels of phenylalanine, increased excretion of tyrosine and alteration in the serum and urinary tyrosine: leucine ratio. Therefore the dysregulation of phenylalanine and tyrosine metabolism (both CFS patients and RDC/TDM type 1a patients) indicates a potential dysregulation of catecholamine metabolism in the kidney in these chronic pain groups. A reduction in serum tyrosine is associated with a reduction in kidney dopamine levels (Muhlbauer et al, 1997) and these data are therefore consistent with the associations between urinary volume and dopamine levels in fibromyalgia patients (Yunus et al, 1993). The urine volume was also associated with alterations in lysine suggesting that nitric oxide production may also be involved (Table 3.32). Dopamine and nitric oxide have antagonistic actions in the kidney (Haynes et al, 1997; Muhlbauer et al, 1997; Dijkhorst-Oei & Koomans, 1998). Increases in dopamine levels or inhibition of nitric oxide production result in water and sodium retention (reviewed in Bachmann & Mundel, 1994; Muhlbauer et al, 1997; Dijkhorst-Oei & Koomans, 1998) and increases in blood pressure. Conversely reductions in dopamine and increases in nitric oxide result in diuresis and loss of sodium (Haynes et al, 1997) consistent with the findings of this study. Interestingly nitric oxide-mediated hypotension is a normal response to endotoxaemia (Allman et al, 1996). The 7-day severity of facial pain in the CFS group was associated with a reduction in serum sodium and chloride (Table 3.16) and serum sodium levels correlated inversely with serum lysine levels (Table 3.22) but was unrelated to tyrosine or phenylalanine levels. These data suggest that increases in nitric oxide and reductions in catecholamine levels may be important in facial pain expression. Whilst this does not suggest that catecholamines are the primary factors involved in the diuresis associated with pain, it is worthy of note that:

1) chronic fatigue syndrome patients (Demitrack et al, 1992) and fibromyalgia patients (van Denderen et al, 1992) have reduced catecholamines;
2) TMD patients have lower blood pressures than controls (de Abreu et al, 1993);
3) drug-associated dysregulation of dopamine metabolism is associated with TMD symptoms (Lynch et al, 1961; Pertoutka et al, 1988); and
4) use of noradrenergic reuptake inhibiting (antidepressant) medication reduces pain expression in TMD patients (Tura & Tura, 1990).

These data are therefore highly supportive of an association between dysregulation of catecholamine metabolism and TMD symptom expression but suggest that their reduction may be more related to failure to turnoff or inhibit the chronic pain response (phase 3 in Figure 3.13) and to some degree alteration in renal fluid, sodium and metabolite loss. These data therefore suggest that there is an enhanced activation of the spinal hyperalgesic response (nitric oxide - phase 2, Figure 3.13) and a reduction in inhibition of the spinal hyperalgesic response (catecholamines - phase 3, Figure 3.13). The dysregulation of the activating and inhibiting factors are thus likely to lead to a persistent hyperalgesic response and hence chronic pain.

Increases in serum lysine levels (Tables 3.31 and 3.32) were associated with increases in facial muscle pain and nausea, and reduction in the serum sodium levels. Reductions in serum sodium levels were associated with increases in nausea and facial muscle pain (Table 3.21) suggesting that the movement of lysine was strongly inversely associated with sodium movements. Similarly, the relative abundance of lysine was the principle factor associated with alteration in the urine volume (Table 3.12). In the CFS patients with facial pain, serum lysine was positively associated with serum ornithine, aconitic acid and tryptophan levels and negatively correlated with serum sodium, glycine, vitamin B12 and ALT. Thus serum lysine is associated with increases in the urea cycle amino acid, ornithine, and the nitric oxide associated citric acid cycle component aconitic acid, and a reduction in sodium suggestive of an inhibition of Na^+K^+ATPase. Serum ornithine and arginine increase during experimental wound healing (Albina et al, 1990), which supports the possibility that a nitric oxide response occurs during the healing response. Lysine, ornithine and arginine (the precursor of nitric oxide) are all competitively transported across cell membranes by common amino acid pumps, such as system y+ (Albina & Mateo, 1995). As lysine and ornithine are highly correlated in this study it is unlikely that lysine is behaving as a competitor in the majority of patients. The strong correlations between lysine and ornithine, and the nitric oxide associated symptoms suggests that the changes in lysine and ornithine are indicative of a nitric oxide mediated process.

Increasing TMD pain severity was associated with increases in excitatory amino acid excretion in both the RDC/TMD type 1a group and the CFS patients (with TMD symptoms) and is consistent with the acute pain model shown in Figure 3.13. The principle factor determining 7-day face pain severity was a reduction in serum sodium levels and this may be a result of excitatory amino acid activation of NMDA receptor activity and inhibition of Na^+K^+ATPase. Pain responses in the central nervous system (Coderre, 1993; Coderre & Yashpal, 1994; Wong et al, 1998) and peripheral tissues (Holthusen & Arndt, 1995; Kang et al, 1995; Lorenzetti & Ferreira 1996) have been associated with the excitatory amino acids involved in NMDA-mediated nitric oxide production. These excitatory amino acid-mediated nitric oxide changes are associated with a decrease in the detection and tolerance thresholds of pain in humans (Thomsen et al, 1996). The primary factor associated with this alteration in the hyperalgesic pain threshold appears to be inhibition of Na^+K^+ATPase activity (Tavalin et al, 1997). Interestingly, inhibition of Na^+K^+ATPase by nitric oxide was dependent upon the redox status of the cell (Sato et al, 1997) and was dependent upon the oxidation or reduction of the SH group on Na^+K^+ATPase. Sato et al (1997) found that the inhibition of Na^+K^+ATPase by nitric oxide was prevented by sulphur reducing agents (reduced glutathione and cysteine). Importantly superoxide (a by-product of neutrophils), combined with nitric oxide potentiated the inhibition of Na^+K^+ATPase. Boldyrev et al (1997) also showed that nitric oxide radicals combined with iron complexes, were the most potent inhibitors of Na^+K^+ATPase and could be inhibited by the amino acid, cysteine. Cysteine reduced the sulphur groups on Na^+K^+ATPase and restored its activity.

In this study, associations were found between TMD pain symptoms and increases in excitatory amino acids, basophils, neutrophils, iron, S-methylcysteine; and reductions in serum sodium, chloride, vitamin B12 levels and the nitric oxide scavenger, haemoglobin. These data suggest that nitric oxide mediated inhibition of Na^+K^+ATPase is involved in TMD pain. It is therefore interesting to note that an increase in nitric oxide is associated with inhibition of the citric acid cycle enzymes that control glutamate removal (oxoglutarate dehydrogenase) and aconitic acid metabolism (aconitase) (Andersson et al 1998). Aconitic and glutamic acid associations with other metabolites were different in the CFS patients with TMD symptoms, compared with those CFS patients without TMD symptoms. Collectively these data are highly supportive of a heterogeneous aetiology of TMD pain that is principally associated with NMDA receptor activation, nitric oxide increases and inhibition of Na^+K^+ATPase activity. Importantly the therapeutic use of the reducing agent, S-adenosylmethionine (from the vitamin B12 pathway), has been associated with reduction in pain scores in fibromyalgia patients when given orally (Tavoni et al 1987; Jacobsen et al, 1991), but not intravenously (Volkmann et al, 1997). Interestingly, S-adenosylmethionine was given as an antidepressant but did not result in an alteration in depression scores however did reduce the number and severity of muscle tender points. No assessment was made of TMD symptoms in the Fibromyalgia subjects

(Tavoni et al 1987; Jacobsen et al, 1991; Volkmann et al, 1997) however Plesh et al (1996) have demonstrated that 75% of fibromyalgia patients have TMD symptoms.

A reduction in serum sodium levels was the principle multiple regression component involved in TMD pain expression and was more significant in females than males. Variation in sodium levels in CFS patients who reported a 7-day face pain response, compared with CFS patients who did not respond, was associated with alterations in 15 different biochemical and blood cell changes (Table 3.22). The reduction in serum sodium levels was associated with increases in serum lysine, platelet count and erythrocyte sedimentation rate (ESR) and reductions in serum creatinine and platelet volume, and urinary levels of succinic acid, (β-alanine, leucine, alanine, valine, glycine, UM15 and phenylacetic acid. Gibala et al (1997) and Wagenmakers (1998) reported that exercise induced fatigue is associated with a reduction in leucine and components in the last third of the citric acid cycle (succinate-oxaloacetate). In this study the reduction in serum sodium was associated with reductions in both succinic acid and leucine suggesting that it may also be associated with muscle fatigability as well as for the regulation of protein turnover (Mortimer & Poso, 1987). Gibala et al (1997) also showed that during initiation of exercise fatigue the increase in leucine and succinate was also associated with an increase in alanine and pyruvate and a reduction in glutamine. Importantly, the reduction in sodium in this study was associated with a reduction in alanine. Sodium levels were:

1) principally associated with alterations in TMD symptoms in the previous 7-days;
2) not correlated with the increase in urinary aspartic and glutamic acids; and
3) not associated with increasing 12-month frequency and severity of facial pain.

These data suggest the existence of a significant dysregulation of the last third of the citric acid cycle at the time of TMD expression, and that the biochemical processes associated with the 12-month frequency and severity of TMD symptoms are distinct from those involved in development of pain within the previous 7-days.

Increasing average pain severity and the 12-month frequency and severity of facial pain were also associated with an increase in neutrophil and basophil counts, the erythrocyte sedimentation rate (ESR) as well as tissue damage marker enzymes, AST and ALT. The CFS patients who reported TMD pain in the previous 7-days had increased neutrophil counts compared with the CFS patients who had no TMD symptoms. Importantly when the control subjects with TMD symptoms were compared with the control subjects with no TMD symptoms they also had an increase in the neutrophil counts. These data indicate that an increase in neutrophil-associated tissue damage occurs with TMD symptoms. These data are similar to the observation of increases in these tissue damage markers in patients with prolonged exercise associated tissue damage (Kayashima et al, 1995; Mena et al, 1996; Bruunsgaard et al, 1997). Bruunsgaard et al (1997) found that alterations in white blood cell and cytokine profiles occurred with different forms of exercise induced damage. Bruunsgaard et al (1997) also suggested that neutrophil recruitment was associated with alterations in catecholamine levels. In the present study, neutrophil accumulation was associated with alteration in tyrosine levels which is highly supportive of the observations of Bruunsgaard et al (1997). Importantly, increases in serum lysine, which were associated with symptoms and biochemical changes consistent with a nitric oxide response were associated with a reduction in ALT levels. This suggests that increases in serum lysine were associated with an increased nitric oxide response and protective of tissue damage. Therefore a reduction in serum lysine and a blunted nitric oxide response was associated with increased ALT and tissue damage (Lui et al, 1998; Vega et al, 1998). This suggests that although catecholamine alterations may be associated with neutrophil recruitment, the nitric oxide response was also blunted in those patients with increased 12-month TMD frequency and severity.

The neutrophil and basophil counts were increased with 12-month TMD symptom expression. This suggested that variation in immune cell products were associated with variation in symptom expression. This association between immune cell products and symptom variation was confirmed in the RNase-L study. Variations in different immune markers (soluble IL2 receptor and IL-6) and RNase-L were associated with different body pain distributions and symptom expression. These data therefore are consistent with the hypothesis that variations in different immune cells and their respective cytokine and immune mediators are associated with differences in pain distribution and symptom expression. In this context, increases in neutrophil (superoxide, etc) and basophil (histamine) compounds are likely to be associated with TMD symptoms, whilst alterations in soluble IL-2 receptor and IL-6 were associated with symptom expression in different areas of the body. Thus increased frequency and severity of TMD symptom expression in CFS patients is associated with increases in immune cell (neutrophils, basophils) products, most likely neutrophil superoxide and histamine, which in turn are associated with increased tissue damage (ALT, AST, GGT). In support of an immune cell association with TMD, was the observation that CFS patients who reported TMD symptoms also had elevated ANA levels, which is strongly suggestive of an interferon associated event (Weber et al, 1994).

In the CFS patients, TMD symptoms were positively associated with increased neutrophil and basophil levels and what appears to be immune cell-mediated tissue damage, ALT and AST. This links TMD symptoms with neutrophil mediated tissue damage and possibly superoxide production (Wagner et al, 1996; Bruunsgaard et al, 1997; Liu et al, 1998). Interestingly the accumulation of neutrophils and the neutrophil induced tissue damage is increased with a blunted nitric oxide response (Bruunsgaard et al, 1997; Liu et al, 1998), but the tissue damage is not mediated by nitric oxide (Wagner et al, 1996). In this study we found that the increase in the neutrophil levels in CFS patients with facial pain was associated with an increase in vitamin B12 (Table 3.44) that was significantly different from that seen in CFS patients without facial pain. Our group (Richards et al, submitted for publication) found this same association between TMD and alterations in vitamin B12 metabolism in another study. Richards et al, (1999) found that an inversion of the association between vitamin B12 and the oxidative bi-product methaemoglobin occurred in CFS patients with TMD, compared with CFS patients without TMD symptoms. Erythrocyte methaemoglobin is a product of the oxidation of the ferrous iron of the haem group of the haemoglobin molecule (Jaffe, 1981) the formation of which is controlled by cytochrome b5 associated NADH-methaemoglobin reductase (Clopton & Saltman, 1997; Chen & Banerjee, 1998). Nitric oxide (Dotsch et al, 1998), bacterial toxins (Kaca et al, 1995) and many drugs (Pirmohamed et al, 1991) can induce MetHb formation. Interestingly Richards et al, (1999) also found that increases in TMD symptom severity were associated with alterations in ferritin, C-reactive protein and ESR suggesting that an infectious agent may be involved. The increase in ESR was also noted in this study with alterations in TMJ pain. The increases in C-reactive protein and the ESR, which are clinical markers of infection, in association with increasing TMD symptoms, supports the findings in this thesis which associates TMD symptoms with increases in infectious events (McGregor et al, 1996). In TMD patients, the combined increase in methaemoglobin, vitamin B12 levels and neutrophil count, suggests that a common factor may underlie this dysregulation of metabolism. Vitamin B12, NADH and cytochrome b5 are all associated with reduction of methaemoglobin by the NADH-methaemoglobin reductase reaction (Clopton & Saltman, 1997; Chen & Banerjee, 1998). Genetic anomalies in methaemoglobin reductase reaction have been report in 1:100 in the normal population (Winslow & Anderson, 1978). This dysregulated enzyme reaction may be due to genetic polymorphism, an environmental or pathogen-related inhibitor or alteration in redox potential (NADH:NAD^+ ratio). These data also support the observation of an association between TMD and dysregulated redox status and proteolysis.

Within the CFS group prolonged facial pain was also associated with an increase in liver based enzyme release and a reduction in the liver toxin conjugate, hippuric acid. These data suggest that the increase in liver enzymes associated with prolonged facial pain is also associated with impairment of liver function which may be associated with neutrophil-mediated tissue damage mechanisms (Wagner et al, 1996; Liu et al, 1998). Importantly an unusual metabolite, S-methylcysteine, was associated with increases in TMD symptoms. S-methylcysteine is not a host metabolite but can be found in a variety of plants, including Allium sativum (Garlic, Onions), Phaseolus vulgaris (Beans) and Cruciferae (Cabbage, Cauliflower, Broccoli). S-methylcysteine is known to inhibit the liver urea cycle enzyme ornithine decarboxylase and the expression of the early response oncogene, c-jun (Takada et al, 1997). In this study the serum ornithine levels and the basophil count were positively correlated with S-methylcysteine suggesting that ornithine decarboxylase may be inhibited by S-methylcysteine. The increase in the detectable plant product, S-methylcysteine, and the reduction in the liver detoxification marker, hippuric acid, and their association with increases in TMD symptoms, strongly suggests that the alteration in liver function is associated with increased 12-month frequency and severity of TMD symptoms. These data also suggest that toxic substances are associated with

TMD symptoms and that alteration in the host's ability to combat or remove toxins may represent an increased susceptibility to the development of TMD symptoms.

Increases in 12-month frequency and severity of facial pain were associated with increased levels of the environmental organochlorine pesticide toxins, Deildrin and DDE. These two organochlorines are lipid soluble and accumulated in lipids within cell membranes and adipose cells. Deildrin has been shown to preferentially accumulate in the brain stem compared with other parts of the brain and to inhibit GABA-a receptors and monoamine receptors (Brannen et al, 1998; Sanchez-Ramos et al, 1998). Therefore Deildrin accumulates in that part of the brain associated with the nuclei which control the trigeminal system. In this study increasing Deildrin levels were strongly associated with increases in TMD symptoms in CFS males. Deildrin accumulation was associated with evidence of a nitric oxide mediated event, increased lipid turnover (ethanolamine) and the accumulation of the non-host s-methylcysteine, suggesting tissue damage. In the CFS group DDE was positively associated with the 12-month frequency of facial pain, earaches, swallowing difficulties and loss of libido as well as illness duration. DDE was also positively associated with increases in serum glucose, s-methylcysteine, ethanolamine, ALT, basophils and the urinary tyrosine: leucine ratio. These data show that DDE accumulation is association with increased phospholipid turnover and tissue damage. These same correlations were not seen in the control subjects. These data strongly show that DDE and Deildrin accumulate in the lipids of the CFS patients as a result of increased phospholipid turnover and are associated with alterations in the basophil count and tissue damage. DDE is known to alter the lipophilic domains of integral proteins within the membrane thereby inhibiting the function of systems such as $Na^{+}K^{+}$ATPase and glucose transport (Cascordi & Ahlers, 1980; Cascordi & Foret, 1991). In this study the strong positive relationship between glucose and DDE supports the observations of Cascordi & Foret (1991). Dunstan et al (1997) reported that increases in DDE were associated with increases in the neutrophil count and reductions in the lymphocyte count supportive of organochlorine accumulation with enhanced tissue damage. Interestingly, Sitarska (1990) found that sublethal levels of DDE caused a decrease in the capacity for bovine macrophages and neutrophils to phagocytose the *Staphylococcus aureus,* which may have implications of TMD due to the possible relationship with toxic *Staphylococcus spp.*. These data suggest that organochlorine accumulation occurs as a result of the disease process and that once accumulated is associated with alterations in TMD symptoms, white cell and metabolic markers indicating a positive association with the development of TMD and histamine mediated allergen reactions.

The biochemical mechanisms associated with tissue damage were different between males and females. In females, the increase in 7-day face pain severity was associated with reductions in serum sodium levels. Fraser et al (1989) found that females are more susceptible to reduced serum sodium and hyponatraemia as a result of an alteration in $Na^{+}K^{+}$ATPase. Arieff et al. (1972, 1976), Arieff & Fraser (1987, 1988) and Ayus et al. (1987, 1988) all reported that otherwise healthy females are very susceptible to neuronal damage in hyponatremic states. Hyponatraemia and altered $Na^{+}K^{+}$ATPase activity are associated with cell membrane alteration in excitable cells and results in cerebral oedema and muscle contractures. Unstimulated, basal sodium uptake by female and male rat neurones was not statistically different. In contrast stimulated sodium uptake in female rat neurones was a significant, 86% greater than in the males. This sodium flux was associated with a reduced high-energy phosphate production (ATP), elevated intracellular inorganic phosphate and intracellular acidosis. In male cells no such alterations were noted. The intracellular sodium clearance rate in the male was 1.5 times faster then in the female. This suggests the some form of inhibition of $Na^{+}K^{+}$ATPase activity may occur in females. Del Castillo et al. (1987) noted that the $Na^{+}K^{+}$ATPase activity varies with the female reproductive cycle and was decreased in neuronal tissue by oestrogen. Oestrogen was found to inhibit liver (Davis et al, 1978) and gut mucosal (Schwarz et al, 1988) $Na^{+}K^{+}$ATPase activity. Similarly, progesterone has been noted to inhibit cardiac muscle (Temma et al, 1983; Labella et al, 1984) and kidney (Lijnen et al, 1985) $Na^{+}K^{+}$ATPase activity. Geary et al (1998) showed that oestrogen altered vascular tone in females by an increased production of nitric oxide, whilst Hayashi et al (1998) found that macrophage nitric oxide synthetase and nitric oxide production was inhibited by oestrogen. Ikejima et al (1998) found that oestrogen potentiated the toxicity of bacterial LPS toward liver Kupffer cells. These data suggest that females would be more susceptible to toxicity mechanisms that require increased $Na^{+}K^{+}$ATPase activity.

Interestingly the usage of oral contraceptives by females with myofascial pain was associated with a higher daily pain score compared with female patients who did not take oral contraceptives (Dao et al, 1998). Dao et al (1998) also showed that contraceptive use reduced the pattern of pain variability noted with the normal menstrual cycle. Thus variation in female sex hormones are likely to be involved in the variation in the pain mechanisms possibly by variation in nitric oxide and Na^+K^+ATPase activity and may therefore contribute to the increased severity of TMD symptoms in females.

Interestingly in this study (Chapter 2) we found an association between TMD symptoms and sciatica. Lui & Sheu (1997) and Lui et al (1997) reported that bacterial LPS is able to alter different isoforms of Na^+K^+ATPase expressed in the sciatic nerve in rats and that this process was associated with nitric oxide. These data also support the association between the inhibition of Na^+K^+ATPase activity by bacterial toxin/nitric oxide-mediated mechanisms and TMD symptoms.

The hyperalgesic response in the trigeminal system and the neck shoulder muscles have been associated with an NMDA mechanism which results in increased jaw muscle electromyographic activity (Broton & Sessle, 1988; Broton et al, 1988; Hu et al, 1993; Yu et al, 1995; Yu et al, 1996; Chiang et al, 1997). This mechanism was associated with alterations in NaCl, KCl and the inflammatory mediator, histamine. In this study we found that jaw muscle pain was associated with increased levels of the NMDA excitatory amino acids, glutamic and aspartic acids, alterations in sodium levels and increases in the basophil number. Basophils, along with tissue mast cells, are an inflammatory cell responsible for the release of histamine. Thus the parameters associated with TMD symptoms in this study are consistent with those required to initiate the trigeminal hyperalgesic and electromyographic events associated with TMD as determined by Sessle and his group (Broton & Sessle, 1988; Broton et al, 1988; Hu et al, 1993; Yu et al, 1995; Yu et al, 1996; Chiang et al, 1997).

Histamine is a known algesic factor and is produced by both serum basophils and tissue mast cells. In this study there was an increase in basophil counts in both males and females and the basophil count was strongly associated with the 12-month frequency and severity of facial muscle pain. The increase in ALT noted in reperfusion injury is also associated with increased histamine and the oxidative stress product, malondialdehyde

(Dzierzkowska et al, 1994). Similarly rat mast cell histamine release was associated with increased malondialdehyde (Masini et al, 1989) confirming the association between histamine and membrane lipid peroxidation. Vendelbo Johansen et al (1997) showed that inhibition of histamine release during acute infectious mononucleosis resulted in a faster normalisation of tissue damage as assessed by abnormal liver enzyme enzymes (ALT, AST). In this study and that of Richards et al (1999) the basophil count, malondialdehyde and ALT are associated with TMD symptoms. These data combined with the findings of (Broton & Sessle, 1988; Broton et al, 1988) suggest that basophil produced histamine may be involved in TMD expression. However an appropriate study is need to confirm these findings. Interestingly staphylococcus and bacterial LPS enhance histamine release by histamine H2 receptor activity. This results in an alteration in cytokine production (increased IL-4, IL-10; decrease IL-12) and a shift in immune balance which may alter susceptibility to infection, allergy reactions and anti-tumour activity (Schroeder et al, 1997; Elenkov et al, 1998). Glucagon which can inhibit this process (Schroeder et al, 1997) is also important in redistribution of amino acids under stress, sepsis and during trauma, and has as a major action; the increase in liver ALT activity (Begum & Datta, 1991). The catecholamines, noradrenaline and adrenaline, also activate ALT (Begum & Datta, 1991). Interestingly the numbers of α-2-adrenergic receptors is elevated in patients with fibromyalgia (Bennett et al, 1991), which may be the result of increased receptor number due to reduced catecholamine availability. Therefore in the serum of TMD patients the increase in the basophil count, ALT and the reduction in tyrosine may represent a dysregulated ability to provide amino acids during increases in body stress.

The Research Diagnostic Criteria for Temporomandibular Disorders (RDC/TMD) requires the assessment of the somatization and depression scores using the SCL-90-R inventory (Le Resche 1982). In chapter 2 and other studies (Buckelew et al, 1986; Clark et al, 1985; Lee & Lee 1989; Pelz & Merskey 1982; Von Korff et al, 1988; Vimpari et al, 1995) a strong association was found between TMD symptoms and SCL-90-R somatization scores but not depression scores. The somatization score was the primary regression variable that determined the difference between both the CFS patients and the RDC\TMD type 1a pain patients and their respective control groups. In the RDC/TMD type 1a patients somatization was the only dimension score to have an increased prevalence in the RDC/TMD type 1a patients (P<0.0001). In this chapter in the CFS patients, somatization was associated with increases in urine volume, total leukocyte count, serum unknown Ph1 and the ESR and reductions in serum sodium, alanine and haemoglobin. Multiple regression revealed that increased urine volume was the primary regression variable. These data clearly show that the somatization dimension scores were associated with most of the biochemical and blood cell factors strongly associated with TMD symptoms. In chapter 2 no major association was found between TMD symptoms and depression dimension scores. In this chapter the changes in biochemical and blood cell parameters associated with the depression dimension scores in either the RDC\TMD type 1a or CFS patients were unrelated to those associated with TMD symptoms. Therefore these data suggest increases in the somatization scores in TMD patients are associated with the biochemical changes associated with TMD symptom expression. These data do not support any major association between depression and TMD symptom expression. An increase in somatization scores in TMD patients should not be interpreted as somatization disorders as they have a distinct biochemical basis.

Antibiotic use at/or around onset was increased in RDC/TMD type 1a pain patients with a sudden illness onset and repeated prescriptions of antibiotics and having taken antibiotics for >3 months duration were increased in CFS patients with TMD symptoms. Antibiotic use in the CFS patients was associated with increases in DDE and with prolonged antibiotic use in the % basophils, % lymphocytes and the serum tyrosine: leucine ratio. Antibiotic use in the CFS patients was also associated with reductions in serum aspartic acid and the neutrophil: lymphocyte ratio. Apart from the increases in DDE and the % basophils, antibiotic use was associated with reductions in factors that were increased with TMD symptom severity. These data suggest that whilst antibiotic use was positively associated with TMD symptom expression, antibiotic use was also associated with a reduction in the factors associated with TMD expression. This strongly suggests that bacterial factors are associated with TMD expression.

In chapter 2 no major association was found between TMD symptoms and prolonged stress and in this chapter the changes in biochemical and blood cell parameters associated with both parameters were different. These data suggest that even though prolonged stress may be weakly associated with TMD symptoms they represent separate biochemical phenomena.

3.6. Conclusions.

This chapter reports the findings that the biochemical events associated with chronic TMD symptoms appear to be associated with an inflammatory mediated/nitric oxide/NMDA mechanism. The resulting tissue damage and hyperalgesic response in the trigeminal system is similar to that determined by Sessle and his group (Broton & Sessle, 1988; Broton et al, 1988; Hu et al, 1993; Yu et al, 1995; Yu et al, 1996; Chiang et al, 1997). Dysregulation of excitatory amino acids, adrenergic precursors, proteolysis/protein synthesis, fluid and sodium balance and inflammatory cell distribution and evidence of tissue damage were noted. These data offer the basis for the biochemical mechanisms responsible for TMD symptoms.

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CHAPTER 4.

4. Staphylococci and TMD.

4.1. Introduction.

The previous chapters have described that RDC/TMD type 1a patients have a distinct set of symptoms that occurs in both control subjects and CFS patients. Patients with RDC/TMD type 1a symptoms have an increased association with infectious events, irrespective of whether they have CFS or another defined condition. Chapter 3 described that the biochemical events associated with chronic TMD symptoms are potentially associated with an inflammatory mediated/nitric oxide/NMDA-linked mechanism associated with tissue damage and a potential hyperalgesic response in the trigeminal system. In addition it was shown that dysregulation of excitatory amino acids, adrenergic precursors, proteolysis/protein synthesis, fluid and sodium balance and inflammatory cell distribution and evidence of tissue damage were involved. Illness duration was found to be associated with depletion of amino and organic acids that are involved in many processes, including the transfer of nitrogen between muscle and liver. These data provide a biochemical basis for the mechanism of TMD symptom expression.

The initial pilot studies suggested that jaw muscle pain might be associated with staphylococcal toxicity (McGregor et al, 1993). First of the morning urine samples were collected (urethral, midstream and last voided). Microscopic examinations for leukocytes and squamous cells were undertaken to assess pyuria and haematuria as evidence of infection. Microbial samples were cultured on blood layer and CLED agar plates in a humidified, CO_2 supplemented, incubator at 37°C. No subject had evidence of haematuria or pyuria, indicating the presence of a current infectious event. Three of the 6 control subjects had no microbial growth whilst the remaining 3 had growth of diptheroids and/or Lactobaccilus. All TMD patients had heavy growths of *Staphylococcus spp*. Bowel contaminant organisms *E.coli* and *Enterococcus spp*. were detected in 4 of the 9 TMD patients. *Staphylococcus epidermidis, Staphylococcus saprophyticus, Staphylococcus hominus* and *Staphylococcus haemolyticus* were isolated from the samples and are members of the Micrococcus subgroup 3 that are known entero-uropathogens. Thus, the urethral microbial flora of the TMD patients was quite different from pain-free control subjects.

Observation of the agar plates showed that many of the staphylococcal species isolated from the urinary tracts of the TMD patients were haemolytic and degraded the red blood cells in the blood agar. Haemolysins are a group of membrane damaging toxins produced by staphylococcal species. To assess the toxic characteristics of these organisms, supernatants from 12 staphylococci were acquired after overnight growth of the organisms in brain heart infusion broth (BHI). The supernatants were filtered to remove all bacteria and the filtrate was applied to chicken renal fibroblast cultures along with fresh BHI as a control. The cultures were observed microscopically for disruption of fibroblast morphology. Filtrate supernants from staphylococci that were haemolytic were observed to significantly disrupt the fibroblast morphology and in the cases of very haemolytic organisms, complete destruction of the fibroblasts was observed. The control BHI broth did not disrupt fibroblast morphology. Thus the toxicity of the staphylococci may be associated with symptom expression in the TMD patients.

Many chronic illnesses have an infectious nature which may result directly from the pathogenic abilities of the pathogen or alternatively the infectious agents may act as cofactors in illness development or be secondary opportunistic infections that aggravate patient morbidity. The aim of this chapter is to investigate the staphylococcus characteristics in chronic orofacial muscle pain patients and their relationship to symptom and biochemical changes, and to assess which role staphylococci may play in disease expression – the primary agent, a cofactor in disease development or simply an opportunistic secondary infection.

4.2. Literature Review.

4.2.1. Overview of Staphylococcus Species.

Staphylococci are organisms that normally grow on the skin and mucous membranes (mouth, upper respiratory tract, intestine, and genitalia) in humans (Prescott et al, 1990). The clustering cocci were first described by Koch in 1878, whilst Oston first used the term "Staphylococcus" in 1883 (Burnett et al, 1976). However the first major description and species determination occurred 1884 when Rosenbach isolated and described the laboratory characteristics of *Staphylococcus aureus* and *Staphylococcus albus*. These two grouping, *Staphylococcus aureus* and *Staphylococcus albus* are now currently divided into two basic subgroups: coagulase-positive (CoPS) and coagulase-negative (CoNS), which consist of many different subspecies as shown in table 4.1.

Staph.aureus has been implicated in many different disease conditions both as a primary and a secondary pathogen. The organism's ability to initiated disease is dependent upon type, number, and route of introduction, toxicity and previous exposure to the organisms. Skin is the most common site of staphylococcal infection and can range from localised lesions (e.g. Boils) to generalised skin infections (e.g. Impetigo). Staphylococcal infections of the lower respiratory tract generally occur as secondary infections that follow viral infections, which lower general immunity. Staphylococcal toxaemia is usually associated with infections, however staphylococcal toxic shock syndrome is associated with primary colonisation of the vagina in females with *Staph.aureus* that produces toxic shock syndrome toxin-1 (TSST-1). Similarly, *Staph.aureus* associated food poisoning occurs with primary gastrointestinal exposure to enterotoxin producing *Staph.aureus* (five types known as A, B, C, D, E).

Table 4.1. List of Staphylococcal species.

No.	Species	No.	Species
1	*Staph.aureus*	10	*Staph.hominis*
2	*Staph.aureus subsp. Anaerobius*	11	*Staph.lugdunensis*
3	*Staph.epidermidis*	12	*Staph.schleiferi*
4	*Staph.capitis*	13	*Staph.auricularis*
5	*Staph.capitis subsp. Ureolyticus*	14	*Staph.saprophyticus*
6	*Staph.caprae*	15	*Staph.cohnii*
7	*Staph.saccharolyticus*	16	*Staph.cohnii subsp. Urealyticum*
8	*Staph.warneri*	17	*Staph.xylosus*
9	*Staph.haemolyticus*	18	*Staph.kloosii*

4.2.2. Staphylococcal toxins

It has generally been considered that CoPS may be pathogenic whilst CoNS are relatively non-pathogenic, which implies that coagulase is a prominent toxin associated with staphylococcal infections (Blair, 1962). Coagulase reacts with prothrombin to form a complex that has the ability to cleave fibrinogen and cause the formation of a fibrin clot, which can inhibit host defence mechanisms (Prescott et al, 1990). This simplistic assessment of pathogenicity fails to take into account the many different virulence factors that may influence pathogenicity. The role of CoNS in clinical disease needs to be re-evaluated, particularly as they are now known to produce many of the toxins and enzymes that were previously only associated with CoPS (Blair, 1965).

Freer & Arbuthnott (1976) defined microbial toxins to be a group of antigenic proteins of microbial origin that are harmful to sensitive hosts or host tissues when administered in relatively small doses. Table 4.2 shows a list of nine groups of the 19 toxins known to be produced by staphylococcus species. Whilst each of these toxins may have individual effects upon host metabolism, most staphylococci produce multiple toxins and exoproteins that may act to potentiate each other and the virulence of the organism. An excellent example of this co-operative effect of staphylococcal toxins is found in the study by De Kimpe et al (1995) who found that staphylococcal lipoteichoic acid and peptidoglycan potentiate each other. Therefore it is important to understand the mode of synthesis and action of staphylococcal toxins and how this may be associated with the disease process (Thelestam, 1983; Rogolsky, 1979).

Table 4.2. Staphylococcal toxins.

Membrane-damaging toxins or haemolysins: α-,β-,γ-, δ- and horse RBC haemolysin
Epidermolytic toxins, 2 types
Enterotoxins, A,B,C,D,E
Toxic Shock Syndrome Toxin (TSST) 2 types
Panton-Valentine leucocidin
Succinic oxidase factor
Staphylococcal pyrogenic exotoxin (PE)
Peptidoglycan
Lipoteichoic acid

(Arvidson, 1983; Turner & Pickhard 1970; De Kimpe et al, 1995)

4.2.2.a. Staphylococcal enterotoxins and toxic shock syndrome toxin

Staphylococcal enterotoxins are best known as the causative agents of staphylococcal food poisoning. They are a series of 5 low molecular weight (25,000 - 30,000) proteins designated as staphylococcal enterotoxin A, B, C, D and E (SEA, SEB, SEC, SED, SEE, respectively) (Bergdoll, 1983). Enterotoxins are associated with the gastrointestinal symptoms of nausea, vomiting, abdominal cramping and diarrhoea. The enterotoxins can induce toxic shock and are associated with alterations in fever and blood pressure (Bergdoll, 1983).

Toxic shock syndrome toxins (TSST-1, TSST-2) are produced by staphylococci and also have a low molecular weight. Toxic shock syndrome (TSS) is characterised by toxic shock, which is a nitric oxide mediated event resulting in sudden onset of high fever, hypotension, headache, profound myalgia, profuse vomiting and diarrhoea, skin rashes and mucous membrane inflammation, neurologic symptoms and multi-organ-dysfunction (Tofte and Williams, 1982). Davis et al (1980) was not able to distinguish between the toxic shock induced by TSST-1 or the enterotoxins (A-E), but did note that enterotoxin C and TSST-1 are able to potentiate each other. Similarly Chow et al (1983) and Clyne et al (1988) found that the membrane damaging toxin γ production by TSST-1 or Enterotoxin producing strains of *Staph.aureus* were more virulent, and that the total symptom presentation in these patients could not be attributed to the enterotoxins alone. This implicated the membrane damaging toxins in multi-organ symptom presentation in these conditions. Crass & Bergdoll (1986) found that CoNS strains are also capable of producing TSST-1 and the enterotoxins. Thus enterotoxins and toxic shock syndrome toxins produced by staphylococci are associated with significant nitric oxide-mediated toxicity in humans and need to be assessed to establish any relationship with chronic muscle pain.

4.2.2.b. Staphylococcal membrane-damaging toxins

Rogolsky (1979) states that there are five commonly observed staphylococcal membrane-damaging toxins (Table 4.3). Turner and Pickhard (1980) also described a horse erythrocyte haemolysin that was distinct from the five membrane damaging toxins.

Table 4.3. Summary of the properties of staphylococcal membrane-damaging toxins.

Toxin	Physico-chemical properties	Biological properties
α-toxin	Molecular weight =34,000. Occurs as polymers (up to 3 units) Arrhenius effect: Heating to 60°C results in inactivation and aggregation whilst heating to 100°C regains part of its activity.	Lethal, dermonecrotic and haemolytic; Rabbit erythrocytes most sensitive as toxin length corresponds to rabbit cell membrane width. *Mode of action*: pore-forming toxin.
β-toxin	Molecular weight = 30,000. Unstable with storage and purification. Inactivated at 60°C. Best activity after cooling overnight – "Hot-Cold" haemolysis.	Lethality unknown. Requires magnesium. *Mode of action*: Phospholipase-C; degrades sphingomyelin in cell membranes Sheep erythrocytes most sensitive. Inhibited by EDTA.
γ-toxin	Two components (I & II) which act synergistically; Molecular weight I = 29,000; II = 26,000; Inactivated at 60oC and by agar.	Lethal, haemolytic to rabbit, sheep, human erythrocytes. It is antigenic. *Mode of action*: unknown but is inhibited by variety of phospholipids and free fatty acids.
δ-toxin	Molecular weight = 2,799. heat stable to 100oC 25 or 26 amino acid spiral - devoid of histidine, arginine, proline, tyrosine, and cysteine.	Lethality unknown. Broad spectrum of haemolytic activity - Human and Horse erythrocytes most susceptible. *Mode of action*: Pore-forming toxin Inactivated by phospholipids, serum fatty acids <C14:0 or >C20:0.
Leucocidin	Two components (F & S) which act synergistically. Molecular weight F = 32,000. S = 38,000.	Toxic to human PMN and macrophages. *Mode of action*: Creation of a potassium ion channel due to membrane lipids perturbation.

(Gemmell & Roberts, 1974; Kapral, 1976a; Füssle et al, 1981; Freer & Arbuthnott, 1983; Gemmell, 1983; Wadstrom 1993; Gemmell & Schumacher-Perdreau, 1986).

α-,β-,γ- and δ-toxins were originally termed haemolysins, their first identified action (Rogolsky, 1979), and later termed "cytolytic toxins" due to their biological activity on cells other than erythrocytes (Bernheimer, 1974). At low dilutions these toxins did not induce cell lysis but induced changes in membrane permeability and are now termed "membrane-damaging toxins" (Rogolsky, 1979). The membrane-damaging toxins mostly act by forming pores through the host cell membrane.

4.2.2.c. Genetic Regulation of Staphylococcal Membrane-Damaging Toxins.

The pathogenicity of staphylococci is under well-regulated genetic control. During the process of infection these regulating systems respond to changes in the bacterial surroundings and adapt the expression of the virulence genes. Understanding the molecular mechanism involved in these regulating systems is therefore essential for a proper understanding of the pathogenicity of the staphylococci (Janzon & Arvidson, 1990).

In staphylococci the production of the ribonucleic acid III activator protein (RAP) co-ordinates the regulation of exoprotein or accessory protein genes and hence colony toxin production and pathogenicity (Balaban & Novick, 1995; Cheung et al, 1997; Lina et al, 1998). This protein is produced in low concentrations and when the cell density reaches an appropriate concentration the RAP levels are then present in sufficient concentration in the surrounding environment to activate the production of accessory proteins (Ji et al, 1995). This is seen in normal cultures of staphylococci where the toxic exoproteins are only produced after the end of the expediential growth phase (Björkland & Arvidson, 1980; Balaban & Novick, 1995a; Novick et al, 1995), when the normal growth phase proteins start to reduce in concentration (Lebeau et al, 1994). Addition of new growth medium to a stationary or exotoxin-producing phase will result in a switch back to the expediential growth phase (Peng et al, 1988). Interestingly the staphylococcal RAP proteins are used by some staphylococci to inhibit or exclude other bacteria (Ji et al, 1997) and therefore appear to act as virulence factors that promote self-toxin production and inhibit toxin production by their competitors.

The RAP protein acts upon a locus on the *Staphylococcus spp.* chromosome called the 'accessory gene regulator' (*agr*), which controls the synthesis of many exoproteins and toxins. This is now a well-characterised global regulatory system that affects the expression of numerous genes (Balaban & Novick, 1995; Novick et al, 1995; Balaban & Novick, 1995a; Cheung et al, 1997; Lina et al, 1998). These include the membrane damaging α- and δ-toxins (Regassa et al, 1992; Arvand et al, 1990; Recsei et al, 1986). *Agr* is a *trans*-activator of a series of exoprotein genes that are expressed during the stationary phase of growth in *Staphylococcus spp.*. *Agr* acts at the level of mRNA by either enhancing the rate of transcription of its target genes or stabilising the transcription of the target genes (Recsei et al., 1986; Peng et al., 1988). *Agr* itself is not involved directly in the temporal aspect of the regulation. Peng et al (1988) cloned the *agr* locus and localised the insertional mutation to be an open reading frame (ORF) encoding a 241 amino acid polypeptide. This ORF was later called *agrA* (Janzon et al., 1989). So far, at least 14 extracellular toxins and enzymes of *Staph.aureus* are regulated by a set of *trans*-acting elements from the *agr* locus. The δ-toxin gene (*hld*) is transcribed from a promoter immediately upstream of the *agr* locus and is positively controlled by *agr*. *AgrA* activation also leads to an increased transcription of the δ-toxin gene (*hld*) which is used by the staphylococcus as an ion channel to transport ions involved in the synthesis or secretion of those extracellular proteins that are regulated by the *hld* RNA (Janzon & Arvidson, 1990). *Agr* RNAII is required for the expression of RNAIII which in turn plays a central role in the regulation of some target genes, including those of α-toxin (*hla*), β-toxin (*hlb*), protein A (*spa*), and staphylococcal enterotoxin B (*seb*+) (Regassa et al., 1992). The *arg* gene associations are very similar in coagulase-negative staphylococcus and *arg* associated proteins from *Staph.epidermidis* have been shown to be able to activate *arg* gene transcription in *Staph.aureus* (Tegmark et al, 1998; Van Wamel et al, 1998). Thus δ-toxin production is a very good measure of *arg* regulated toxin production by all staphylococcus species and plays the dual role of initiating toxin production by the Staphylococcus and acting as a toxin.

The environmental regulation of the accessory regulatory gene and its toxin production are important for the understanding of staphylococcal toxicity. However little is known of the environmental stimuli that alter the *arg* gene. Increasing availability of substrate will reactivate the growth of the organisms and hence reduce *arg* expression (Peng et al, 1988), whilst increasing cell density and increased RAP will activate *arg* expression (Lina et al, 1988). Importantly a reduction in osmolarity (low sodium levels) in the medium is associated with an increase in toxin production and *arg*-associated RNA whilst an increase in extracellular sodium results in inhibition of arg expression (Regassa & Betley, 1993). This may be of extreme importance in TMD patients as a positive association was found between facial muscle pain and reductions in serum sodium levels. Therefore the understanding of the molecular mechanisms governing the *agr* gene and its expression is essential for the understanding of staphylococcal toxin-associated diseases.

4.2.2.d. Staphylococcal δ-toxin in disease pathogenesis.

Wiseman (1970) suggested that δ-toxin was of no consequence in the pathogenicity of staphylococcal disease since its action was inhibited *in-vitro* by normal serum. It may be difficult to ascribe a particular role of δ-toxin in the pathogenesis of staphylococcal disease as it is a major factor associated with the *arg* gene and consequently the production of many different toxins (Janzon & Arvidson, 1990). Kapral et al. (1976a; 1976b) demonstrated that *Staph aureus* δ-toxin inhibited water absorption in rabbit jejunum and ileum and in guinea pig ileum via increased cyclic adenosine 3',5'-monophosphate (cAMP) activity, which is similar yet different from cholera toxin (O'Brien et al, 1978). This effect was shown to be blocked by lecithin, a known inhibitor of δ-toxin (Rogolsky, 1979). Scheifele et al. (1987) found an association between CoNS δ-toxin and neonatal necrotizing enterocolitis. This is a serious bowel disorder that principally affects infants in intensive care settings, which supports the pathogenicity of δ-toxin *in-vivo*.

Kasimir et al. (1990) reported that δ-toxin altered human neutrophil functions by exerting pro-inflammatory effects. Increases were noted for neutrophil: 1) intracellular calcium concentration; 2) the generation of oxygen radicals; 3) the release of enzymes; and 4) the activation of the acetyltransferase, leading to the formation of platelet activating factor (PAF). δ-toxin also has the ability to induce Tumour Necrosis Factor (TNF) production by monocytes and to potentiate the effects bacterial LPS and TNF-α on neutrophils (Schmitz et al, 1997; Veldkamp et al, 1997), even at low non-toxic levels. Veldkamp et al (1997) were not able to attribute these effects to δ-toxin when used alone, which did not eliminate the potentiation effect reported by Schmitz et al (1997). This ability of δ-toxin to potentiate the effects of other compounds to activate neutrophils may be of importance as the long-term severity and frequency of jaw muscle pain was positively associated with increased neutrophil levels and evidence of tissue damage (Chapter 3). Richards et al (1999) also showed that an increase in markers of oxidative radical generation was associated with TMD symptom expression.

Despite doubt placed on δ-toxins role in pathogenicity, recent research has shown that it is a membrane-damaging toxin that should be re-examined. It has been shown to be involved in intestinal diseases and to have effects on human neutrophil functions. Its broad spectrum of cell-damaging activity makes it a very important candidate as a virulence factor in localised staphylococcal infections and it may be an important aetiological agent of diseases such as CFS and chronic pain.

4.2.3. Staphylococci, Toxicity and Chronic Pain.

4.2.3.a. Neutrophils and Nitric oxide.

Pilot study data of the urinary bacterial species in chronic orofacial muscle pain patients (n=8) and sex matched controls revealed an increased carriage of midstream urinary *Staphylococcus spp.* without haematuria or pyuria (McGregor et al, 1992). The staphylococcal human red blood cell haemolysins or δ-toxins produced by the staphylococcal species is a group of highly lipid soluble small polypeptides of low immunogenicity (Nolte & Kapral 1981) which have been reported to:

1) form cation selective voltage-dependent and -independent ion channels (Mellor et al, 1988);
2) cause cell membrane lipid disturbances (Molby, 1983; Rydall & MacDonald, 1992);
3) cell permeabilization (Umezawa et al, 1980);
4) reduce epidermal growth factor receptor function (Kasimir et al, 1990);
5) activate Phospholipase A2 (Kasimir et al, 1990);
6) induce the production of prostaglandins and platelet activating factor (PAF) (Kasimir et al, 1990); and
7) act as an enterotoxin (Overturf et al, 1990).

In vitro experiments have displayed many of the attributes of δ-toxin, but very few studies have implicated these toxins as having effects in vivo (Overturf et al, 1990). In summary, the mechanisms of action of δ-toxin clearly associate it with potential dysregulation of proteolytic events within the host, including cytokine, cell volume and growth hormone dysregulation. These data suggest that lipid soluble staphylococcal membrane damaging toxins may have a role in the aetiology of chronic orofacial muscle pain.

Chapter 3 described the TMD symptoms and the pain response association with alterations in liver enzymes and other measures suggestive of a toxicity reaction, not unlike a low-grade toxic-shock response. This reaction appeared to be related to an enhanced nitric oxide response, a reduced catecholamine reaction and an increase in excitatory amino acid concentrations.

De Kimpe et al (1995) and Kengatharan et al (1998) reported that the increase in gram positive bacterial toxic shock, which has increased since 1980, was associated with the production of cell wall components such as lipoteichoic acid and peptidoglycan.

Staph.aureus cell wall lipoteichoic acid administered to rats induced a nitric oxide response. The nitric oxide response was potentiated by *Staph.aureus* cell wall peptidoglycan (De Kimpe et al, 1995; Kengatharan et al, 1998). *Staph.aureus* lipoteichoic acid and peptidoglycan injection resulted in a significant fall in mean arterial blood pressure, arterial oxygen tension, pH, bicarbonate and the noradrenaline pressor response, and increases in tachycardia, urea, creatinine and markers of tissue injury, creatine kinase, bilirubin, ALT and AST (De Kimpe et al, 1995; Kengatharan et al, 1996). Injection of either compound alone did not evoke the same response. Staphylococcal induced nitric oxide production is mediated by tumour necrosis factor-α (TNF-α) or interferon-γ (IFN-γ) (Sasaki et al, 1988). The incidence, rate of development, and severity of staphylococcal arthritis were greater in inducible nitric oxide deficient mice compared with control mice (Sakiniene et al, 1997; McInnes et al, 1998). These mice had an increased production of tumour necrosis factor-α (TNF-α), interferon-γ (IFN-γ) and interleukin-4 (IL-4), showing that interactions between staphylococcus and nitric oxide are important in cytokine-mediated polarisation of the immune system. Thus a nitric oxide-mediated toxicity reaction can be induced by staphylococcal products and is mediated by and may induce a change in cytokine levels.

Prolonged exposure of staphylococci to the nitric oxide radial (.NO) results in a dose-related loss of viability which is not as effective as the neutrophil produced superoxide radical (O^{2-}) (Kaplan et al, 1996). Superoxide and .NO initially together result in a reduction in superoxide-mediated staphylococcal death during the early phases of the killing process but enhances the rate of loss of staphylococcal viability as the process continues. Importantly, inhibition of inducible NO synthase substantially decreased the neutrophil killing capacity of *Staphylococcus aureus* (Malawista et al, 1996). Staphylococcal induced nitric oxide production and staphylococcal death is mediated by TNF-α or IFN-γ (Sasaki et al, 1988). Thus alteration in nitric oxide production is important not only for symptom expression but is also used for host defence against pathogens.

4.2.3.b. Staphylococcus, Basophils and Histamine.

Sakiniene et al (1997) and McInnes et al (1998) revealed that an alteration in cytokine production was associated with staphylococcal products. There was a switch from a balanced immune reaction to one dominated by increased IL-4 and IL-10, and decreased IL-12, which appear to be mediated by nitric oxide mediated processes and staphylococcal products (Schroeder et al, 1997; Elenkov et al, 1998). The shift in immune response is associated with a shift toward increased susceptibility to infection, allergies and anti-tumour activity. Chapter 3 described the positive association between the basophil count, facial pain expression, bladder infections and diarrhoea (Table 3.24), as well as alterations in urine volume and the total amino acid excretion (Table 3.25). These data strongly suggest a nitric oxide mediated alteration in immune balance. In support of this Cleveland et al (1992) reported that chronic rhinitis was strongly associated with fibromyalgia. Cleveland et al (1992) showed that 50% of the subjects with rhinitis had diffuse aching pain with more than 11 fibromyalgia points and had an increased reactivity to inhaled allergens. These data suggest that a histamine based alteration in immune function and staphylococcal toxicity may play a significant role in TMD expression. This is supported by the findings of Sessle and his group (Broton & Sessle, 1988; Broton et al, 1988; Hu et al, 1993; Yu et al, 1995, Yu et al, 1996, Chiang et al, 1997), who found that histamine was able to induce jaw muscle hyperactivity and hyperalgesia.

Staphylococcus aureus has been linked with the cause of allergies and asthma, and has been shown to induce a dose-response histamine release from basophils in human airway tissues (Clementsen et al, 1991). A similar event has also been observed with *Staph epidermidis* peptidoglycan which induced histidine decarboxylase (the enzyme forming histamine), in the liver, lung, spleen and bone marrow, and ornithine decarboxylase in the tissues except for the lung (Ando et al, 1994). Clementsen et al (1990a) also showed that viruses, such as Influenza A virus, can enhance basophil histamine release induced by *Staphylococcus aureus* and that this process was a sugar-mediated reaction (Norn et al, 1990; Clementsen et al, 1990b). It has subsequently been proposed that a combination of *Staphylococcus.spp* and an allergen can induce an exaggerated histamine response histamine in asthmatic patients (Oehling et al, 1997). Thus a combination of viral infections and staphylococcus toxins may be able to induce exaggerated responses and that these may be involved in TMD symptom expression. These events may explain the increased prevalence of TMD symptoms in CFS (Chapter 2) and fibromyalgia patients (Plesh et al, 1996).

4.3. METHODS.

4.3.1. RDC\TMD type 1a patients.

4.3.1.a. Patient and Control Selection.

Forty-six patients sequentially presenting for orofacial pain management, complaining of orofacial muscle pain, were recruited as previously described in Chapter 2. These patients were designated to be the RDC/TMD type 1a patient group. Forty-one age and sex matched control subjects (C) were recruited, as previously described in Chapter 2. Each patient and control subject provided informed consent in accordance with ethics requirements (Universities of Sydney and Newcastle) and was assessed by the author.

All subjects completed a CPRU questionnaire and a Hopkins Symptom Check List-90-Revised (SCL-90-R), as previously described in Chapter 2. Muscle pain was confirmed by palpation of superficial facial, neck and shoulder and muscle pain. Symptom severity were assessed using the visual analogue pain scale of average pain intensity and the SCL-90-R question responses (Questions 1, Q4, Q12, Q14, Q27, Q39, Q40, Q42, Q52, Q55, Q56, Q58, Q66), as previously described in chapter 2. Each subject collected the first morning urine specimen on the day of the second visit. The urine was processed using the methods described in Chapter 3.

4.3.1.b. Microbial Specimen Collection and Processing.

Nose, throat, and for the female participants, vagina swabs were collected and transported in Start's medium (Transystem, Interpath Services P/L), and cultured on Columbia horse blood agar (Oxoid). After incubation for 24 hours at 35^0C, all cultures were examined under a stereomicroscope (Zeiss, Germany) for different morphological types of staphylococcal isolates. A single colony representing a morphological type was streaked onto horse blood agar and incubated at 35^0C for a further 24 hours. A sample of a minimum of three colonies of a morphological type from each site was used to establish adequate representation. Cultures with a positive Gram and catalase reaction were then identified according to their biochemical characteristics.

4.3.1.c. Microbial Identification.

The identification of staphylococcal isolates was performed by the methods of Kloos & Bannerman (1995), Herbert et al. (1988), and Kloos & Schleiffer (1975). Carbohydrates in purple agar base (Difco) and other test reagents were prepared, sterilised and added to 8 wells x 12 strips of a microtitre plate. Each strip of 8 wells was prepared as follows: 1) 5μL of a sterile 20% carbohydrate solution was placed in the first six wells, 2) 100µL each of urease and nitrate solution was transferred to the remaining two wells, and 3) 95µL of sterile purple agar was added to the first six wells with the carbohydrate solution and mixed gently. Each well of each row contained a different carbohydrate/reagent solution. A solution of staphylococcal culture in saline prepared to a turbidity of a 0.5 MacFarland was used as the inoculum. The six carbohydrates tested in each strip were: lactose, maltose, trehalose, mannitol, sucrose and mannose. Only strains that were susceptible to furazolidone (100µg) and resistant to bacitracin (0.04U) were included in this study (Herbert et al, 1988). A tube coagulase with citrated rabbit plasma was used to differentiate the *Staph.aureus* group from the CNS group. A positive coagulase reaction with human plasma and a positive ornithine decarboxylase activity were used for the confirmation of *Staph.lugdunensis* (Bes et al, 1989). Resistance to the antibiotics novobiocin (5µg) and polymyxin B (300U) were included in the differentiation of *Staph.saprophyticus* and *Staph.epidermidis* respectively from other related species (Herbert et al, 1988). Similarly, bacitracin (10U) was included to help differentiate *Staph.haemolyticus* from other novobiocin susceptible staphylococci (Herbert et al, 1988). All equivocal identifications were confirmed with an API Staph kit (Bio Merieus, France).

4.3.1.d. Staphylococcal Supernatant

Staphylococcal strains were cultured in 5mL of brain heart infusion broth (Oxoid, England) at 350C for a minimum of 18 hours. Cultures were centrifuged at 25,000g at 40C for 20 min and each supernatant was filtered through a 0.22µm membrane filter (Millipore, Bedford, USA). Each strain was tested for toxic shock syndrome toxin (TSST) , enterotoxins A-D (SEA-SED) and haemolysin production.

4.3.1.e. Detection of TSST-1 and SEA-SED

Staphylococcal TSST-1 and enterotoxin types A, B, C, and D were detected by the reverse passive latex agglutination kits (SET, and TSST-RPLA Oxoid, England), used as microslide agglutination tests described by Murrell et al (1993). Each test sample was accompanied by positive and negative known controls. Agglutination was observed with phase contrast microscopy.

4.3.1.f. Assay for Haemolytic Activity

Rabbit, sheep, horse and human type 'O' red blood cells were washed three times in 1mM MgPBS (0.5M KH2PO4, 0.5M KH2PO4, 1mM MgSO4) and a final 1%(v/v) of each of the four red cell types was prepared. An equal volume of the sterilised staphylococcal supernatant was added to each of the four 1% (v/v) red cell pellets. The suspensions were mixed and incubated at 35^0C for 30 min. The 'hot-cold' effect of β-haemolysin on sheep red cells was demonstrated by a further incubation at 40^0C for 1 hr. The 'heat stable' effect of δ-haemolysin was demonstrated by heating the staphylococcal supernatant at 80^0C for 15 minutes prior to incubation with human red cells (Molby, 1983). The 'heat stable' effect of 'horse'-haemolysin was also investigated. After incubation, all suspensions were centrifuged for 5 min and the absorbency of each supernatant determined at 541 nm (the frequency of light absorbed by haemoglobin). A sample substituting deionised water for MgPBS of each of the four red cell types was measured at neat to represent 100% haemolysis and at 1:2 and 1:4 dilutions. Haemolytic activities of samples were calculated from a standard curve.

4.3.1.g. Definition of positive (significant) haemolysis.

Positive haemolysis for each of the four haemolysins is defined as a value greater or equal to two standard deviations (SD) above the mean haemolysis produced by staphylococcal strains from control subjects, thus representing a high value seen in only 2.5% of a normally distributed population. The mean haemolysis percentage and SD for the four haemolysins produced by staphylococcal isolates from control subjects were: for α-haemolysin, 2.6% and 7.8% respectively ; β-haemolysin , 10.1% and 19.7%; δ-haemolysin, 0.7% and 1.2%; and 'horse'-haemolysin, 2.5% and 2.2%. Positive (significant) haemolysis for each of the four haemolysins was therefore defined as: >18.1% for α-haemolysin; >49.5% for β-haemolysin; >3.1% for δ-haemolysin; and >6.9% for 'horse'-haemolysin. Negative haemolysis for each of the four haemolysins included all values that were less than two SD above the mean haemolysis of control staphylococcal strains.

4.3.1.h. Staphylococcal Indices.

Several staphylococcal indices were calculated for each subject: the total number of staphylococcus isolates, the total number of toxin-producing staphylococcus isolates, the total number of CoNS isolates and the total number of toxin producing CoNS isolates. The 4 haemolysins were assessed as the mean percentage haemolysis per subject, the mean percentage haemolysis of the CoNS isolates, the mean percentage haemolysis of the CoNS isolates multiplied by the number of CoNS isolates, and the mean percentage haemolysis of the CoNS isolates multiplied by the number of toxin-producing CoNS isolates. These haemolysin indices were calculated to more accurately reflect the potential toxin load per subject.

4.3.2. CFS Microbiological, Cytokine and RNase-L Study.

Forty-two CFS patients who complied with the Fukuda definition (Fukuda et al, 1994) and 50 community-based age- and sex-matched healthy control subjects were recruited as described in chapter 3. University of Newcastle Ethics approval was obtained. Patients completed a CPRU questionnaire that obtained scalar responses (0-4) to 86 questions on physical, cognitive and psychological symptoms over the preceding 7 days. This questionnaire is used as a standard questionnaire for poly-symptomatic patient symptom assessment.

Blood samples were collected and transported to a commercial laboratory for assessment of RNase-L activity and cytokine markers (sIL-2r, IL-6) (Melbourne Forensic Diagnostic Laboratories, Melbourne, Australia) as previously reported in Chapter 3. Nasal swabs were taken from all subjects at interview and the staphylococci were identified, cultured and the percentage haemolysis assessed as per sections 4.3.1.b through to 4.3.1.g.

4.3.3. Statistical Analysis.

All percentage data were arcsine transformed before analysis and other data not having a normal distribution were log transformed for usage in logistic regression analysis. Subject characteristics and symptom incidence was assessed using chi-square probability and students t-tests. Correction for multiplicity occurred where necessary. Univariate group differences were assessed on untransformed data using the non-parametric Mann Whitney U test and Spearman rank correlation, and ANOVA. Group differences were further assessed using multivariate standard and forward-stepwise discriminate function analyses, Pearson product moment analyses on the transformed data (significance $P<0.05$; NS = not statistically significant). Odds ratios were calculated using the logistic regression (Quasi-Newton) estimation method from within the statistical package. These data were processed using Access97 TM (Microsoft, Redmond, WA, USA), Excel97 TM (Microsoft) and Statistica TM (Ver. 5.1, Statsoft, Tulsa, OK, USA).

4.4. Results

4.4.1. RDC/TMD type 1a Patients.

4.4.1.a. Patient and control subjects characteristics.

Of the 46 RDC\TMD type 1a pain patients and 41 control subjects, 43 RDC\TMD type 1a pain patients and 40 control subjects completed all questionnaires and examination requirements as reported in Chapter 2. From these two clinical groups, thirteen MP patients and seven control subjects were further excluded as urine samples were either not obtained (n=12) or related to laboratory problems (n=4). The demographics of the study population were: number MP=29, C=34: age MP=38.2±13, C=35.0±13; gender characteristics (% Female) MP=83%, C=68%; marital status (% Married) MP=48%, C=47%.

4.4.1.b. Urinary Metabolites Excretion Patterns

Thirty-six urinary metabolites were detected and measured in this study. The results of these analyses identified in this study were presented in Chapter 3.

4.4.1.c. Staphylococcal Colonisation in RDC/TMD type 1a pain patients.

The RDC\TMD type 1a pain patients and control subjects were screened for the carriage of staphylococcal species. Each isolate was assessed for the ability to produce membrane-damaging toxins as described in the materials and methods. Table 4.4 shows that the control subjects had a higher carriage prevalence of *Staphylococcus aureus* compared with the RDC\TMD type 1a pain patients. In contrast the RDC\TMD type 1a pain patients had a higher carriage incidence of CoNS compared with the control subjects. The RDC\TMD type 1a pain patients also had a higher incidence of carriage of significant toxin-producing CoNS (as defined in the materials and methods) compared with the control subjects. As there was no difference in the total number of staphylococcal isolates detected in both the clinical and control groups, it can be concluded there is an alteration in the distribution of the staphylococcal population in the "commensal" flora of the RDC\TMD type 1a pain patients. These changes in the bacterial flora reflect a shift towards an increased colonisation of toxin producing organisms in the RDC\TMD type 1a pain group.

Multivariate analyses were then used to assess whether these "commensal" distribution parameters were different between RDC\TMD type 1a pain patients and control subjects. Discriminant function analysis revealed that the RDC\TMD type 1a pain patients and control subjects had different staphylococcal profile characteristics ($P<0.0003$, Table

4.4) and that the primary discriminant variable was indeed the number of toxin-producing CoNS species per subject.

Table 4.4. Staphylococcal characteristics for the RDC\TMD type 1a pain patients (n=29) and control subjects (n=34) and the Spearman rank correlation with pain severity (VAS) in all study subjects (n=63).

Nasal coagulase positive staphylococci (CoPS)				Pain Severity	
	TMD	**C**	***P***	**r**	***P***
Isolates/subject	1.4(4)	3.2(5)	NS		NS
Species/subject	0.1(0.4)	0.4(0.5)	<0.02	-0.28	<0.03
Nasal coagulase negative staphylococci (CoNS)					
Isolates/subjects	1.83(0.5)	1.38(0.9)	<0.02	0.38	<0.003
Total Species/subject	2.3(1.0)	1.8(0.8)	NS		NS
Toxin-producing Species/subject	1.3(0.7)	0.7(0.9)	<0.004	0.51	<0.00002
% Toxin-producing CoNS of Total CoNS	68(33)%	38(45)%	<0.001	0.39	<0.002
Males N=16					
Toxin-producing Species/subject	1.3(0.8)	0.7(1.3)	NS	0.38	NS
% Toxic CoNS of Total CoNS	70.0(44)%	27.3(41)%	NS	0.46	NS
Females N=45					
Toxin-producing Species/subject	1.3(0.6)	0.6(0.6)	<0.0006	0.56	<0.00007
% Toxin-producing CoNS of Total CoNS	67.4(64)%	45.5(66)%	NS	0.35	<0.02

Discriminant function analyses	**Discriminant function analysis**
TMD vs Controls	**Pain severity / bacterial parameters**
Wilks' λ = 0.630; F(3,40)=7.820, P<0.0003	Wilks' λ = 0.445; F(12,95)=2.854, P<0.003
Discriminant variables:	**Discriminant variables:**
1) Toxin-producing CoNS	1) Toxin-producing CoNS
2) Genital CoNS species	2) CoNS
3) CoPS	3) Nasal CoNS species

Statistical methods: Forward stepwise discriminant function; t-test; Spearman rank-order correlation, $\alpha = P<0.05$.

The odds ratio for carriage of nasal staphylococcal species per subject comparing the RDC/TMD type 1a patients and the control subjects was 1.8 (χ^2=0.23, P=0.63) and not statistically significant. The RDC\TMD type 1a pain patients had an increased odds ratio for carriage of toxin-producing CoNS compared with the control group (OR=9.2, χ^2=12.96, P<0.0004). The odds ratio for carriage of toxin-producing CoNS when comparing the control subjects who reported no musculoskeletal symptoms (asymptomatic control group; n=8) with the RDC\TMD type 1a pain patients was 21.7 (χ^2=10.47, P<0.002). The odds ratio for carriage of toxin-producing CoNS when comparing the control subjects who reported no musculoskeletal symptoms (asymptomatic control group; n=8) with the remaining control subjects was 2.9 (χ^2=1.45, P=0.23) and therefore not statistically significant. The odds ratio for carriage of toxin-producing CoNS when comparing the control subjects who reported musculoskeletal symptoms (remaining control group; n=26) with the RDC\TMD type 1a pain patients was statistically significant at 7.4 (χ^2=9.275, P<0.003). Thus patients with facial muscle pain had a significantly increased likelihood of carriage of toxin-producing CoNS.

In Chapter 2 there was a significant increase in the reporting of pain in the partners of the RDC/TMD type 1a patient group (Sen=39.5%, Spec=90%; P<0.02). When the study subjects in long-term relationships were compared, no difference was found in the total staphylococcal species per subject (RDC/TMD=1.8, C=1.7), total isolates per subject (RDC/TMD=1.9, C=1.8) or the prevalence of carriage of CoNS (RDC/TMD=94%, C=96%). There was however an increase in the odds ratio of prevalence of carriage of toxin-producing CoNS (OR=5.8, χ^2=5.062, P<0.03), and an increase in the number of toxin-producing CoNS species per subject (RDC/TMD=1.2±0.66, C=0.65±0.65 – P<0.008). The RDC/TMD type 1a patients who reported pain in their partners, also had a reduction in the number of CoPS (RDC/TMD=0.18±0.39, C=0.52±0.51 – P<0.03) and the number of toxin-producing CoPS (RDC/TMD=0, C=0.35±0.49 – P<0.006). In the RDC/TMD type 1a patients who reported pain in their partners, there was an increased odds ratio for the reporting of neck pain (OR>100, c^2=5.7, P<0.02) and low back pain (OR>100, c^2=4.3, P<0.04) in their partners. Thus the RDC\TMD type 1a patients, who reported that their long-term partners had pain, had an increased carriage of toxin-producing CoNS.

4.4.1.d. Toxin Producing Staphylococcus and Pain Severity (VAS)

The RDC\TMD type 1a pain group had a mean VAS score of 2.8±1.0 (±SD), as distinct from the control group which had a zero score. Discriminant function analysis of the total study population (n=63) revealed that increasing pain severity scores were associated with alterations in the incidence of "commensal" staphylococcal isolates (P<0.003), and that the primary correlate for pain severity was the prevalence of toxin producing CoNS species per subject (Table 4.4). Univariate correlation analysis revealed that increasing pain severity, as assessed by a VAS, was positively associated with the incidence of toxin producing CoNS (P<0.00002), the incidence of CoNS isolates and the proportion of toxin-producing CoNS species carried per subject. Conversely, increasing pain severity was negatively associated with the number of CoNS species. A positive correlation was specifically identified between pain severity and the mean percentage CoNS "horse"-toxin per isolate (r=0.35, P<0.006), whereas negative correlations were observed between the mean percentage α-toxin (r=-0.26, P<0.04) and β-toxin (r=-0.39, P<0.002) producing CoNS with pain severity.

4.4.1.e. Toxin Producing Staphylococcus, Illness Duration, Body Pain Distribution, Symptom prevalence and Age.

Table 4.5 shows the univariate analyses of the associations between the staphylococcal parameters with illness duration, body pain distribution, symptom prevalence and age. The carriage of toxin-producing CoNS in the nose was positively associated with

Table 4.5. Spearman rank correlation analysis of the staphylococcal parameters with illness duration, body pain distribution, symptom prevalence and age.

Staphylococcal Parameter	Duration	Body Distribution	Symptom Prevalence	Age
Nasal Toxic CoNS species/subject	0.51 <0.001	0.46 <0.0002	0.28 <0.03	-
Mean % Horse haemolysis/isolate	0.41 <0.02	0.39 <0.003	-	-
Total CoNS isolates	0.43 <0.007	0.40 <0.002	-	-
Max % Horse Haemolysis/subject	0.36 <0.03	0.37 <0.003	-	-
CoNS/subject	-	0.38 <0.003	-	-
Max % α-haemolysis/subject	-	-	-	0.31 <0.02
CoPS/subject	-0.39 <0.02	-0.29 <0.04	-	-
Toxic CoPS/subject	-0.51 <0.002	-	-	-

illness duration, body pain distribution and symptom prevalence, but not age. The carriage of the toxin-producing CoNS was positively associated with the pain parameters, whilst the carriage of CoPS was negatively associated with pain parameters. Thus the carriage of toxin-producing CoNS was strongly correlated with increases in pain severity and distribution.

The odds ratios for carriage of a (total and species) toxin-producing staphylococcus were calculated for the various body regions and are shown in Table 4.6. These data reveal

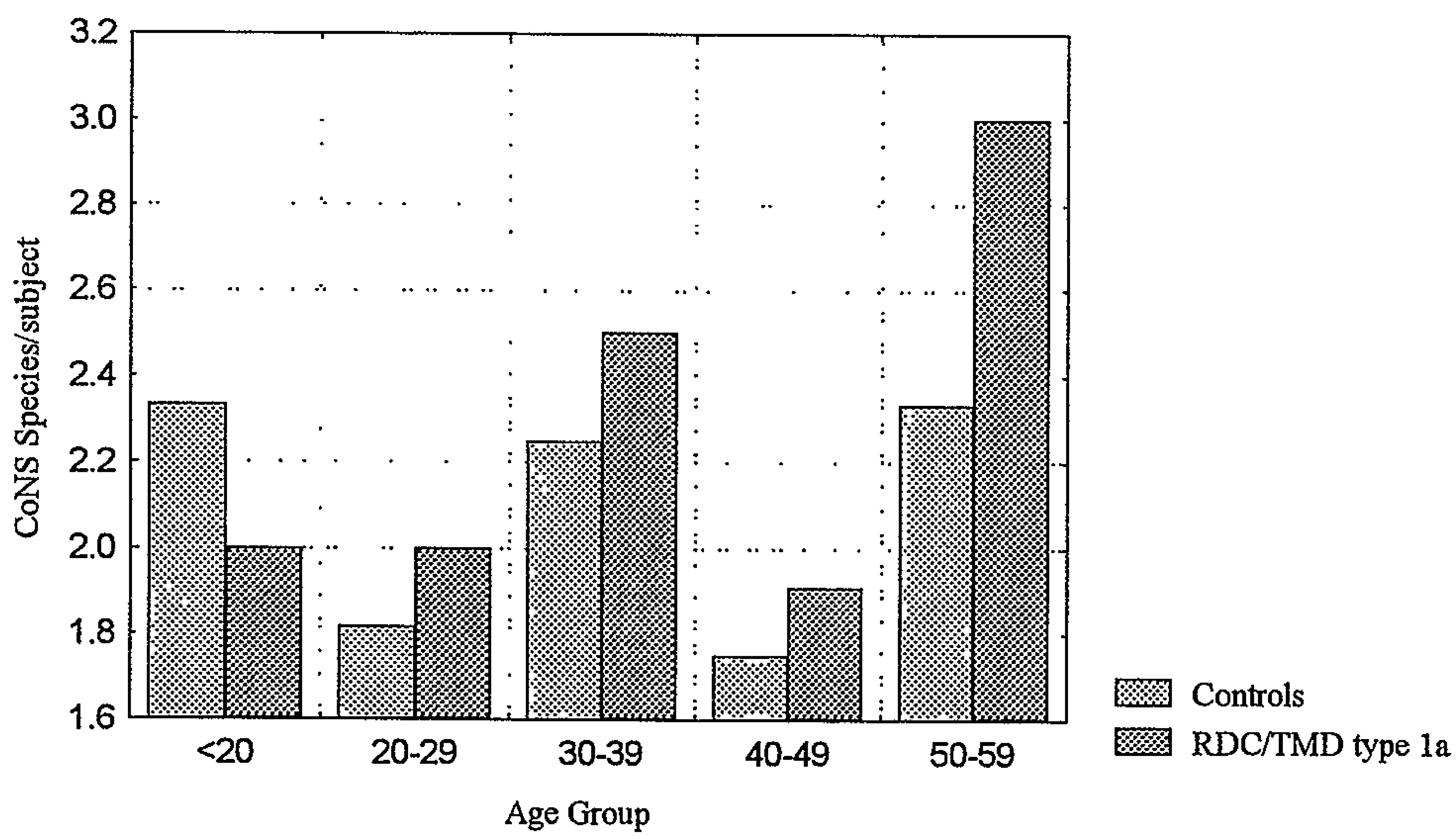
Table 4.6. Odds ratio analysis of toxin-producing CoNS (total and individual species) and body pain distribution.

Body Area	Odds Ratio			Odds Ratios for the toxin-producing CoNS Species			
	OR	χ^2	*P*	Species	OR	χ^2	*P*
Face	7.4	16.9	<0.00004	*Staph.epidermidis*	4.4	5.7	<0.02
Head	8.2	15.8	<0.00007	*Staph.epidermidis*	4.1	6.3	<0.02
				Staph.xylosis	4.1	4.4	<0.04
Low back	16.1	13.9	<0.0002	*Staph.lugdunensis*	8.9	9.6	<0.002
				Staph.xylosis	4.2	4.5	<0.04
Neck	7.1	13.7	<0.0003	*Staph.xylosis*	4.6	5.1	<0.03
				Staph.epidermidis	3.3	4.7	<0.03
Arm	6.3	13.0	<0.0004	*Staph.lugdunensis*	5.9	5.9	<0.02
				Staph.xylosis	3.9	4.0	<0.05
TMJ	7.7	10.1	<0.002	*Staph.hominis*	4.8	6.6	<0.01
Leg	5.3	8.2	<0.005	*Staph.lugdunensis*	4.3	4.5	<0.04
Shoulder	3.3	6.1	<0.02	Nil			
Abdomen	4.4	4.6	<0.04	*Staph.lugdunensis*	9.0	9.3	<0.003
Anterior Chest	2.4	1.8	NS	Nil			

that a significant increase in the typical body pain pattern described for myofascial pain syndrome associated with an increased carriage of toxin-producing CoNS. Facial, head, neck, arm and low back muscle pain were strongly associated with toxin-production by CoNS. These data also show that different toxin producing CoNS species are associated with variation in body pain distribution. *Staph.epidermidis* was strongly associated with facial, head, neck muscle pain whilst *Staph.lugdunensis* was associated with low back, arm, leg and abdominal pain. *Staph.xylosis* was associated with head, neck, arm and low back pain whilst *Staph.hominis* was associated with TMJ pain. Thus variation in the different species of CoNS is associated with variation in body pain distribution whilst multiple carriage of toxin-producing CoNS was associated with increased body distribution of pain (Table 4.5).

Figure 4.1 and 4.2 shows the age distribution of carriage of the CoNS and toxin-producing CoNS species per subject, respectively. The levels of carriage of toxin-producing CoNS in the facial pain patients were more pronounced in the individuals less than 40 years of age.

Figure 4.1. Age distribution of carriage of CoNS species per subject in the RDC/TMD type 1a pain patients and control subjects.



There was no significant variation in CoNS or toxin-producing CoNS between any of the age-groups. Figure 4.3 shows the CoPS and CoNS bacterial counts/ml on the agar plates in relationship to age. This graph clearly shows that the proportion of CoNS of the total staphylococcus species increase during the periods of peak incidence of TMD whilst the proportion CoPS declines during that period. The age of peak incidence of TMD 30-40 years of age also was the age of the highest CoNS colony count/ml.

Figure 4.2. Age distribution of carriage of toxin-producing CoNS species per subject in the RDC\TMD type 1a pain patients and control subjects.

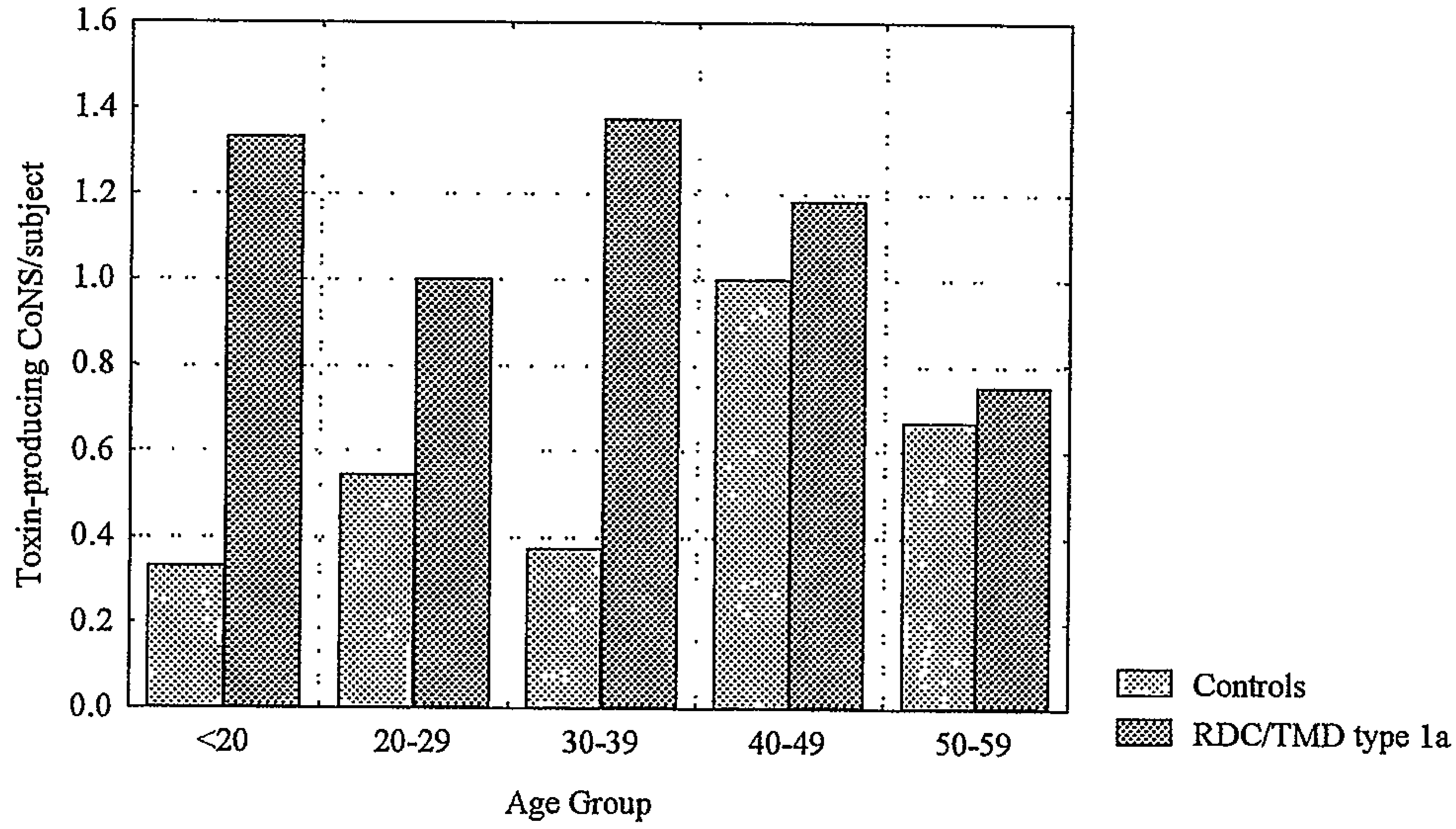
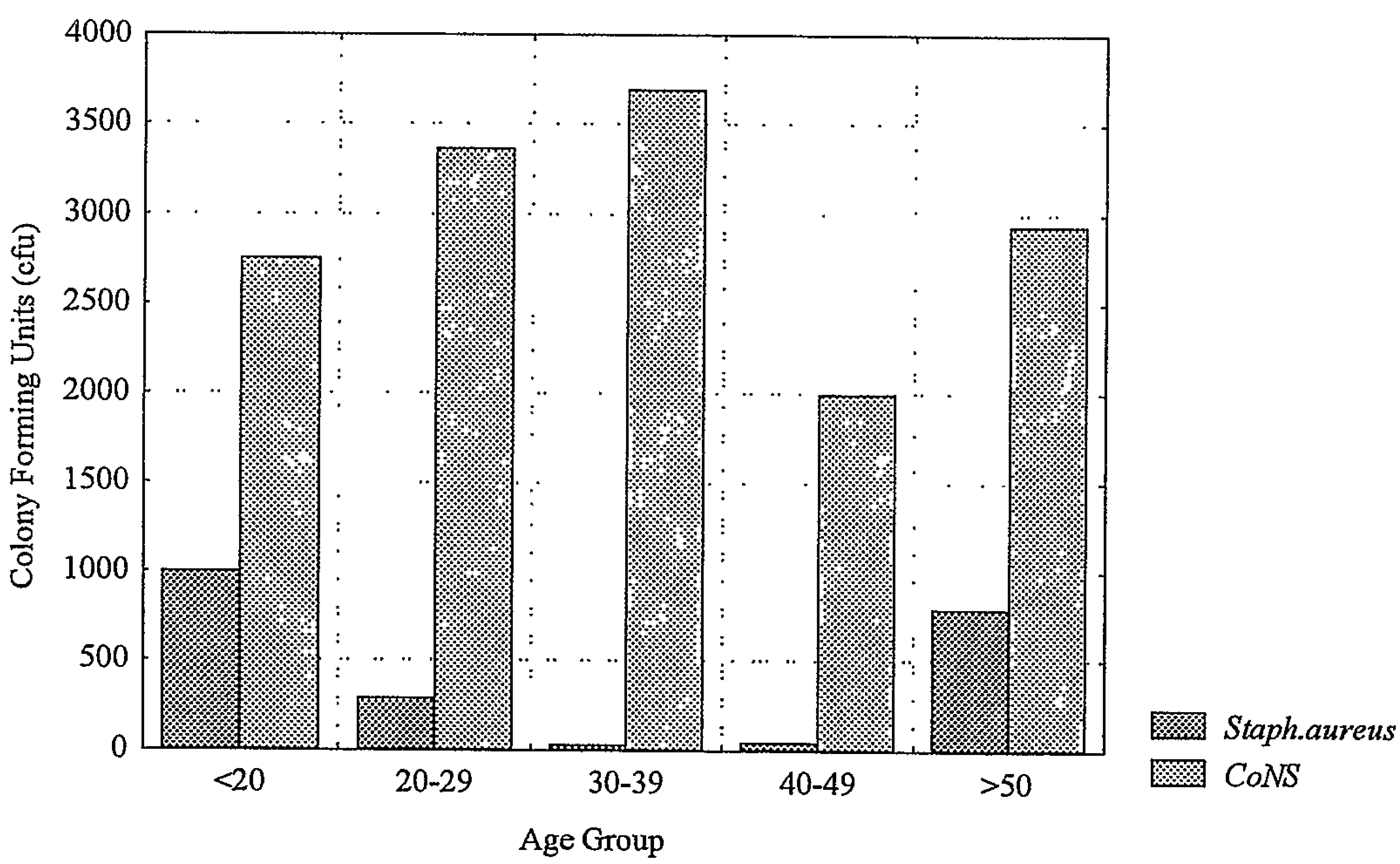


Figure 4.3. CoPS and CoNS colony forming unit count in the total study population in relationship to age.



4.4.1.f. Toxin Producing Staphylococcus and Urinary Metabolite Changes.

Forward stepwise multiple regression analyses of the study population (n=63; Table 4.7) revealed that the incidence of toxin-producing CoNS per subject was associated with alterations in the concentration ($P<0.003$) and relative abundance ($P<0.002$) of urinary metabolite excretion profiles. UM28 was the primary correlate in the regression model. Univariate regression showed strong positive correlation between the incidence of CoNS and

UM28, and the tyrosine: leucine ratio (Table 4.7), and a negative correlation with leucine, indicating an association between CoNS toxicity and the marker ratio for proteolysis: protein synthesis.

In the RDC\TMD type 1a pain patients (n=29; Table 4.7) forward stepwise multiple regression analyses also suggested the association of toxin-producing CoNS and the alterations in the concentration (P<0.003) and relative abundance (P<0.008) of the urinary metabolite excretion profiles. An unknown urinary metabolite, UM13a, together with the excitatory amino acids, glutamic and aspartic acid were included amongst the 3 primary factors associated with the carriage of toxin-producing CoNS. Univariate regression analyses revealed positive associations between the prevalence of toxin-producing CoNS and glutamic acid in both the concentration and relative abundance analyses. In the concentration analysis, positive correlations were also found for the carriage of toxin-producing CoNS with the following unknown urinary markers: UM15a, UM13a, UM27 and UM13. These data indicate a positive association between the urinary excretion of the excitatory amino acids and a series of unknown metabolites and CoNS toxicity in the RDC\TMD type 1a pain patients.

Table 4.7. Correlation of the urinary metabolite excretion with the number of toxin-producing CoNS within the whole study group (n=63), and the RDC\TMD type 1a pain group (n=29).

Group Characteristic	**Concentration (mMol)** r value	P value	**Relative Abundance** r value	P value
All subjects (n=63)				
UM28	0.34	<0.007	0.30	<0.02
Tyrosine: Leucine Ratio	0.36	<0.004		
Leucine	-0.31	<0.02	-0.32	<0.02
Multiple regression analysis	R^2=0.325, F=3.72, P<0.003 *Primary correlates:* 1) UM28 (P<0.001) 2) hippuric acid (P<0.13) 3) UM15a (P<0.11)		R^2=0.420, F=3.29, P<0.002 *Primary correlates:* 1) UM28 (P<0.003) 2) UM14 (P<0.06) 3) 3-methylhistidine (P<0.05)	
MP only (n=29)	r	P	r	P
Glutamic acid/glutamine	0.45	<0.02	0.43	<0.03
UM15a	0.44	<0.02	-	-
UM13a	0.42	<0.03	-	-
UM27	0.38	<0.04	-	-
UM13	0.37	<0.05	-	-
Multiple regression analysis	R^2=0.693, F=4.76, P<0.003 *Primary correlates:* 1) UM13a (P<0.02) 2) glutamic acid (P<0.14) 3) aspartic acid (-P<0.01)		R^2=0.672, F=3.69, P<0.008 *Primary correlates:* 1) glutamic acid (P<0.05) 2) aspartic acid (-P<0.01) 3) leucine (-P<0.09)	

*Statistical method: Spearman rank-order correlation, $\alpha = P$<0.05.

4.4.1.g. Toxin Producing Staphylococcus and Sudden Onset.

There was no significant difference in any other staphylococcal parameter between the RDC\TMD type 1a patients who reported a sudden onset compared with a gradual onset. There was a much higher odds ratio for carriage of toxin-producing CoNS in those reporting a gradual onset (OR>25, χ^2=16.68, P<0.00004), compared with those reporting a sudden onset (OR=2.92, χ^2=5.37, P<0.02). No difference between the sudden and gradual onset patients could be determined by multiple regression using the staphylococcal parameters.

4.4.1.h. Toxin Producing Staphylococcus and Antibiotic Use at Onset.

Table 4.8 shows that the RDC/TMD type 1a patients who reported antibiotic use at the time of onset of their illness had higher CoNS colony counts and increased mean and maximum δ-toxin production rates compared with those RDC/TMD type 1a patients who did not report antibiotic use at onset. Discriminant function analysis revealed a difference in the staphylococcal characteristics between the RDC/TMD type 1a patients reporting antibiotic use at onset and the remaining RDC\TMD type 1a patients. The mean percentage δ-toxin haemolysis per isolate was the only variable selected by regression analysis. There was a much higher odds ratio for carriage of toxin-producing CoNS in those reporting antibiotic use at onset (OR>25, χ^2=11.99, P<0.0006) compared with those not reporting antibiotic use at onset (OR=13.8, χ^2=9.64, P<0.002). Thus antibiotic use at onset of the illness was associated with an increase in the δ-toxin-producing CoNS found in RDC/TMD type 1a patients.

Table 4.8. Differences in the staphylococcus characteristics between RDC/TMD type 1a patients who reported antibiotic use at onset compared with those who did not report antibiotic use at onset.

Parameter	**Antibiotics**	**No Antibiotics**	***P*-Value**
Patient Characteristics			
Age	34.9±14.5	42.8±10.1	NS
% Female	100%	71.4%	NS
Staphylococcus Characteristics			
CoNS colony count	1839±3323	956±2734	<0.04
Maximum % δ-toxin Haemolysis/Subject	24.9±17.7	5.7±8.8	<0.0005
Mean % δ-toxin Haemolysis/Isolate	13.8±9.5	3.3±4.4	<0.002
Discriminant Function Analysis	Wilk's λ = 0.595, F=14.96, P<0.0008		
	Primary correlate:		
	1) Mean δ-toxin CoNS Haemolysis/subject P<0.0008		

4.4.1.i. Toxin Producing Staphylococcus and SCL-90-R Somatization and Depression Scores.

Spearman Rank correlation analysis revealed a positive correlation between SCL-90-R somatization and the CoNS colony count (r=0.33, $P<0.02$). No other staphylococcal parameter correlated with either the SCL-90-R somatization or depression scales. Neither odds ratio or regression model analysis revealed an association between toxin-producing CoNS and the SCL-90-R somatization or depression dimension scores.

4.4.1.j. Toxin Producing Staphylococcus and Symptoms.

The carriage of toxin producing CoNS was associated with increased odds ratios for 14 different symptoms, as shown in Table 4.9. The carriage of a toxin producing CoNS was also associated with an increased odds ratio for the reporting of constant pain but not irregular or intermittent pain as well as an increased likelihood of reporting muscle tenderness on use and with palpation, and with cold weather associated pain. Toxin producing CONS were also associated with the inability to identify a cause of pain, onset of pain in the facial muscles and a sore throat at onset.

Table 4.9. Associations between toxin-producing CoNS and general body symptoms, modifying events and onset associated events.

Parameter.	Odds Ratio		
Symptoms	OR	χ^2	*P*
Muscle fatigue	22.0	16.3	<0.00005
Morning joint stiffness	16.4	12.6	<0.0004
Paraesthesia	>100	10.9	<0.001
Earaches	>100	7.9	<0.005
Irritable bowel	8.8	6.6	<0.01
Abdominal bloating	5.2	5.3	<0.02
Palpitations	4.4	5.4	<0.02
Cervical lymphodynia	4.4	5.4	<0.02
Arthritis	6.1	4.1	<0.05
Hair loss	6.1	4.1	<0.05
Sinus problems	4.2	3.8	<0.05
Diarrhoea	4.2	3.8	<0.05
Hyperaesthesia	4.2	3.8	<0.05
Dizziness/Faintness	3.6	3.9	<0.05
Pain type/Influence			
Muscle tenderness with use	15.4	10.0	<0.002
Constant pain	>100	9.2	<0.003
Muscle pain worse with cold	>100	9.2	<0.003
Muscle tenderness on palpitation	>100	5.9	<0.02
Onset events			
No obvious onset event	>100	5.0	<0.03
Onset in facial muscles	>100	4.3	<0.04
Sore throat at onset	6.5	3.8	<0.05

4.4.2. RNase-L Study Group.

4.4.2.a. Group Differences.

Table 4.10 shows that there was no difference in age or sex between the CFS and control subjects. The CFS patients had an increased prevalence of facial pain and TMJ clicking or locking. CFS patients also had an increased prevalence of patients reporting a single TMD problem, as well as those reporting combined facial pain and TMJ clicking/locking. Thus this cohort of CFS patients had an increased prevalence and severity of TMD symptoms which is similar to that seen in the Royal North Shore Hospital CFS study group.

4.4.2.b. Association between Staphylococcal Species and CFS.

Table 4.10 also shows that CFS patients did not have any difference in carriage of CoPS, CoNS or toxin-producing strains or species of CoNS. However the CFS patients did

Table 4.10. Differences in the study subjects characteristics, TMD parameters and staphylococcus characteristics between the CFS patients and control subjects.

Parameter	*CFS*	*Control*	*P-Value*
Patient Characteristics			
Age	42.6±15.0	37.4±11.6	NS
% Female	73.8%	84.0%	NS
Number	42	50	
TMD Prevalence/Severity	n(%)	n(%)	
Number reporting face pain	20 (47.6)	3 (6.0)	<0.0001
Number reporting TMJ Clicking/locking	13 (31.0)	7 (14.0)	<0.05
Number of TMD symptoms	n(%)	n(%)	
0	17 (40.5)	41 (82.0)	<0.0001
1	17 (40.5)	8 (16.0)	<0.009
2	8 (19.0)	1 (2.0)	<0.007
Staphylococcus Characteristics			
CoPS isolates/subject	0.31±0.64	0.18±0.44	NS
CoNS isolates/ subject	2.60±1.60	2.74±1.60	NS
Toxic CoNS isolates/ subject	0.83±0.76	0.72±0.67	NS
Maximum % Haemolysis/Subject			
α-toxin	30.09±30.28	24.28±25.53	NS
β-toxin	14.90±18.30	7.54±8.66	<0.007
δ-toxin	26.95±24.93	16.26±15.15	<0.02
Horse RBC-toxin	21.79±14.84	19.10±15.22	NS
Mean % Haemolysis/Isolate			
α-toxin	18.70±21.68	11.76±15.32	NS
β-toxin	9.69±14.49	4.81±5.89	<0.009
δ-toxin	15.50±13.49	8.46±7.97	<0.003
Horse RBC-toxin	14.29±10.39	10.79±8.50	NS

show an increase in the maximum percentage haemolysis produced for the highest toxin-producing organism carried per subject and as the mean percentage haemolysis per isolate for both the membrane damaging toxins: β-toxin and δ-toxin. The odds ratio for carriage of all CoNS was 1.3 (χ^2=0.129, P=0.72) whilst for the carriage of toxin-producing CoNS was 1.4 (χ^2=1.471, P=0.23), which indicates that CFS patients do not have an increased likelihood of carriage of toxin-producing CoNS species. There was a high odds ratio for carriage of β-toxin producing strains in CoNS but it was not statistically significant (OR=>100, χ^2=2.835, P=0.09), which resulted from the very few β-toxin-producing CoNS strains identified (n=2). No significant odd ratios were found for carriage of CoNS toxin-producing species for any of the other individual membrane-damaging toxins (all OR's<1.3).

4.4.2.c. Association between Staphylococcal Parameters and Facial Pain.

Table 4.11 shows the forward stepwise multiple regression analyses comparing the RDC/TMD type 1a pain CFS patients against: 1) all other subjects and; 2) the control subjects using total and toxin producing CoPS and CoNS. The jaw muscle pain patients had increased maximum percentage δ- and β-toxin haemolysis per subject compared with the remaining study subjects. The jaw muscle pain patients also had increased mean percentage δ- and horse-toxin haemolysis per CoNS isolate compared with the remaining study subjects. Multiple regression analysis revealed that the number of toxin producing CoNS isolates per subject was strongly associated with jaw muscle pain.

The odds ratio for carriage of a toxin-producing CoNS species/subject (all toxins included) in the facial pain patients was 3.1 (χ^2=2.233, P=0.14), whilst for the carriage of combined δ- horse-toxin-producing CoNS species per subject was 6.5 (χ^2=4.492, P<0.04), which confirms the findings seen in the analysis of the defined RDC/TMD type 1a pain patient group. Thus toxin-producing CoNS carriage is strongly associated with:

1) patients defined to have RDC/TMD type 1a pain; and
2) facial pain expression within a defined CFS cohort.

This suggests that the same toxin producing CoNS strains were associated with jaw muscle pain in both a precisely diagnosed group and within a group who simply reported their responses by questionnaire.

Table 4.12 shows the Spearman rank correlation analysis of the association between the 7-day scalar severity score (0-4) for face pain and the toxin and species parameters in all study subjects.

Table 4.11. Differences in the subject and staphylococcus characteristics between facial pain patients and subjects without facial pain.

Parameter	**Facial pain**	**No Facial pain**	***P*-Value**
Patient Characteristics			
Age	42.7±15.4	36.7±10.0	NS
% Female	82.6%	83.0%	NS
Number	23	40	
TMJ Clicking/Locking	n(%)	n(%)	
Prevalence	9 (39.1%)	6 (12.8%)	<0.02
Severity	1.00±1.51	0.26±0.79	<0.009
Staphylococcus Characteristics			
CoPS isolates/ subject	0.17±0.43	0.17±0.49	NS
CoNS isolates/ subject	3.17±1.44	2.70±1.57	NS
Toxin-producing CoNS isolates/ subject	1.13±0.76	0.68±0.66	<0.02
Maximum % Haemolysis/Subject			
α-toxin	28.94±24.44	23.53±25.83	NS
β-toxin	12.98±14.05	7.62±8.93	<0.03
δ-toxin	31.42±26.24	15.02±14.24	<0.002
Horse RBC-toxin	24.02±13.22	18.27±15.21	NS
Mean % Haemolysis/Isolate			
α-toxin	13.97±11.53	11.64±15.78	NS
β-toxin	6.77±5.35	4.90±6.06	NS
δ-toxin	16.21±11.51	7.89±7.64	<0.002
Horse RBC-toxin	15.28±10.31	10.23±8.34	<0.04
Multiple Regression Analysis			
Face v all remaining subjects	**Face v Controls only**		
$R^2=0.150$, $F=9.662$, $P<0.003$	$R^2=0.189$, $F=2.793$, $P<0.05$		
Primary correlates:	*Primary correlates:*		
1) Toxin-producing CoNS $P<0.003$	1) Toxin-producing CoNS $P<0.03$		
	2) CoNS $P<0.16$		
	3) Toxin-producing CoPS –$P<0.26$		

Table 4.12. Spearman rank correlation analysis of the staphylococcal parameters with the 7-days severity scores for face pain and TMJ clicking/locking in all study subjects (n=63).

Measure	**α-toxin**	**β-toxin**	**δ-toxin**	**Horse-toxin**
Facial Pain				
Maximum Haemolysis/ subject	0.01	0.04	0.23	0.09
Toxin positive species/ subject	-0.04	-0.03	0.22	0.05
Toxin positive isolates/ subject	0.29 <0.03	0.29 <0.03	0.30 <0.02	0.30 <0.02

CoNS Isolates	**CoNS Species**	**δ-,Horse-toxic Species**	**α-,β- toxic Species**
0.34 <0.006	0.23	0.37 <0.005	0.18

TMJ Clicking/locking	**α-toxin**	**β-toxin**	**δ-toxin**	**Horse-toxin**
Maximum Haemolysis/ subject	0.02	0.12	0.08	0.04
Toxin positive species/ subject	0.03	0.06	0.10	0.08
Toxin positive isolates/ subject	0.12	0.12	0.13	0.14

CoNS Isolates	**CoNS Species**	**δ-.Horse-toxic Species**	**α-,β- toxic Species**
0.16	0.16	0.12	0.16

The number of toxin positive isolates per subject for all toxins, the total CoNS isolates and, δ- and horse-toxin-producing CoNS species were positively correlated with face pain severity. Thus the carriage of toxic CoNS is associated with the prevalence and severity of RDC/TMD type 1a pain in CFS patients and is consistent with the staphylococcal toxicity changes seen in the RDC\TMD type 1a pain study. Figure 4.4 and 4.5 show the age-associated carriage of the toxin-producing CoNS and proportion of toxin-producing CoNS in the patients with facial pain compared with the remaining study subjects. Thus the TMD patients have higher numbers and proportions of toxin-producing CoNS compared with the remaining study subjects.

Figure 4.4. Difference in carriage of toxin producing CoNS between patients with facial pain compared with the remaining study subjects in relationship to age.

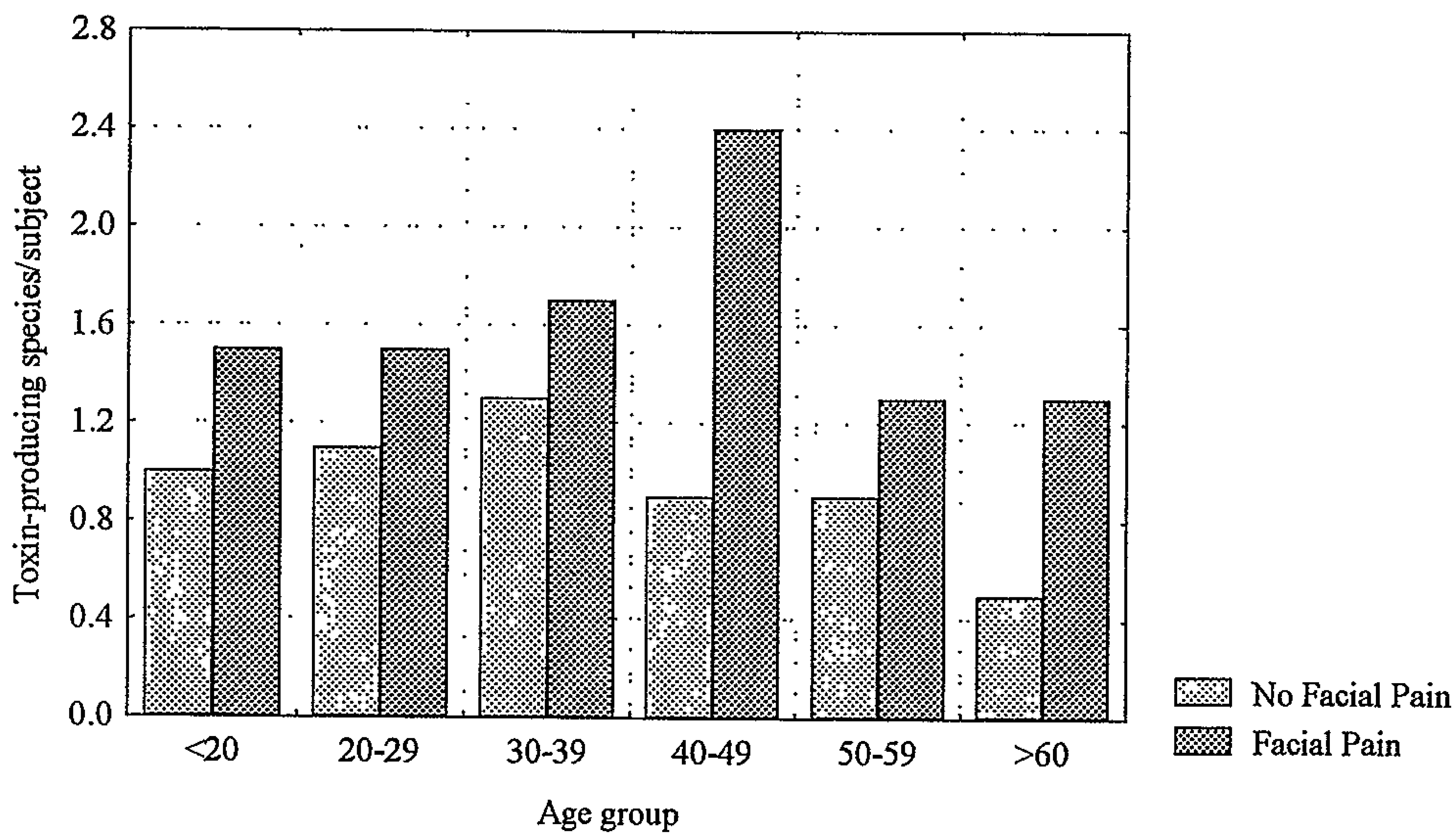
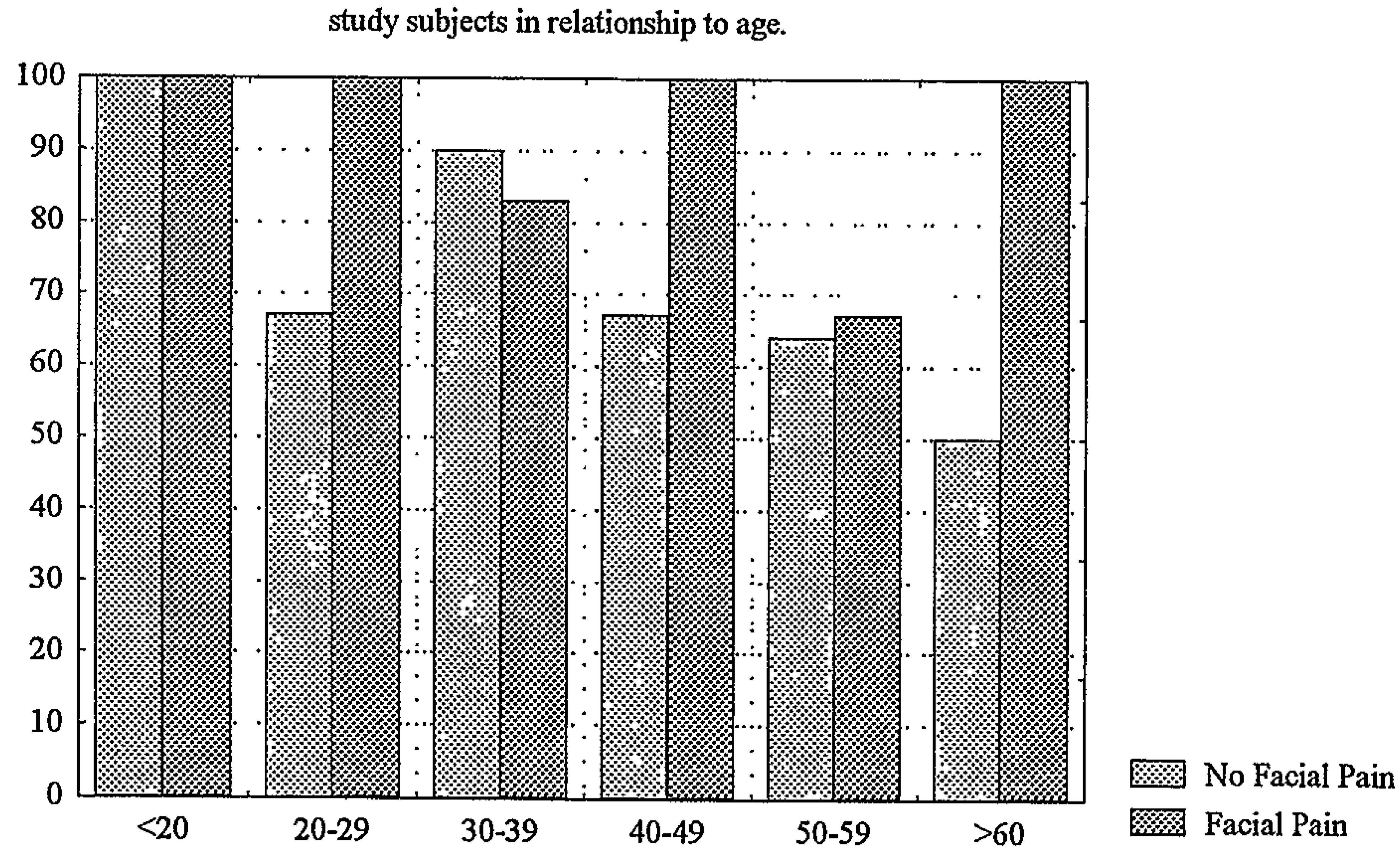


Figure 4.5. Difference in the proportion of toxin-producing CoNS between patients with facial pain compared with the remaining study subjects in relationship to age.



4.4.2.d. Association between Staphylococcal Parameters and TMJ Clicking/Locking.

Tables 4.12 and 4.13 show the uni- and multi-variant analyses for those subjects who were reported TMJ clicking/locking compared with the remaining study subjects.

Table 4.13. Differences in staphylococcus characteristics between TMJ clicking/locking patients and control subjects.

Parameter		TMJ Click/Lock	No TMJ Click/Lock	P-Value
Patient Characteristics				
	Age	36.5±12.3	38.5±11.8	NS
	% Female	82.6%	81.4%	NS
	Number	20	43	
Facial Pain		n(%)	n(%)	
	Prevalence	9 (45.0%)	2 (4.7%)	<0.0001
	Severity	1.05±1.36	0.47±0.21	<0.0001
Staphylococcus Characteristics				
	CoPS isolates/ subject	0.30±0.66	0.14±0.35	NS
	CoNS isolates/ subject	2.75±1.44	2.84±1.56	NS
	Toxic CoNS isolates/ subject	1.00±0.73	0.74±0.69	NS
	Maximum % Haemolysis/Subject			
	α-toxin	25.70±25.6	27.61±29.1	NS
	β-toxin	13.64±15.1	11.86±16.3	NS
	δ-toxin	20.47±13.9	21.97±24.1	NS
	Horse RBC-toxin	21.41±10.9	20.99±16.9	NS
	Mean % Haemolysis/Isolate			
	α-toxin	13.55±13.8	14.60±19.4	NS
	β-toxin	7.80±7.5	8.13±14.0	NS
	δ-toxin	12.83±10.6	11.41±11.1	NS
	Horse RBC-toxin	14.22±8.8	12.62±9.4	NS
Multiple Regression Analysis				
TMJ v all remaining subjects		**TMJ v Controls**		
R^2=0.036, F=2.053, P<0.16		R^2=0.088, F=1.643, P<0.21		
Not statistically significant		Not statistically significant		

The patients reporting TMJ clicking or locking had an increased prevalence and severity of facial muscle pain. No staphylococcal parameter was associated with TMJ clicking or locking.

4.4.2.e. Association between Staphylococcal Parameters, RNase-L, sIL-2r and IL-6.

There was no association between any staphylococcal parameter and RNase-L, sIL-2r or IL-6 levels when assessing the entire study group, the CFS patients or the control subjects. However, when the study subjects were divided into those subjects with facial pain and the remaining subjects, significant positive correlations in the remaining subject group were found between RNase-L and the maximum percentage δ-toxin per subject (r=+0.36, P<0.02), and the mean percentage haemolysis per isolate for:

1) δ-toxin (r=+0.41, P<0.005);

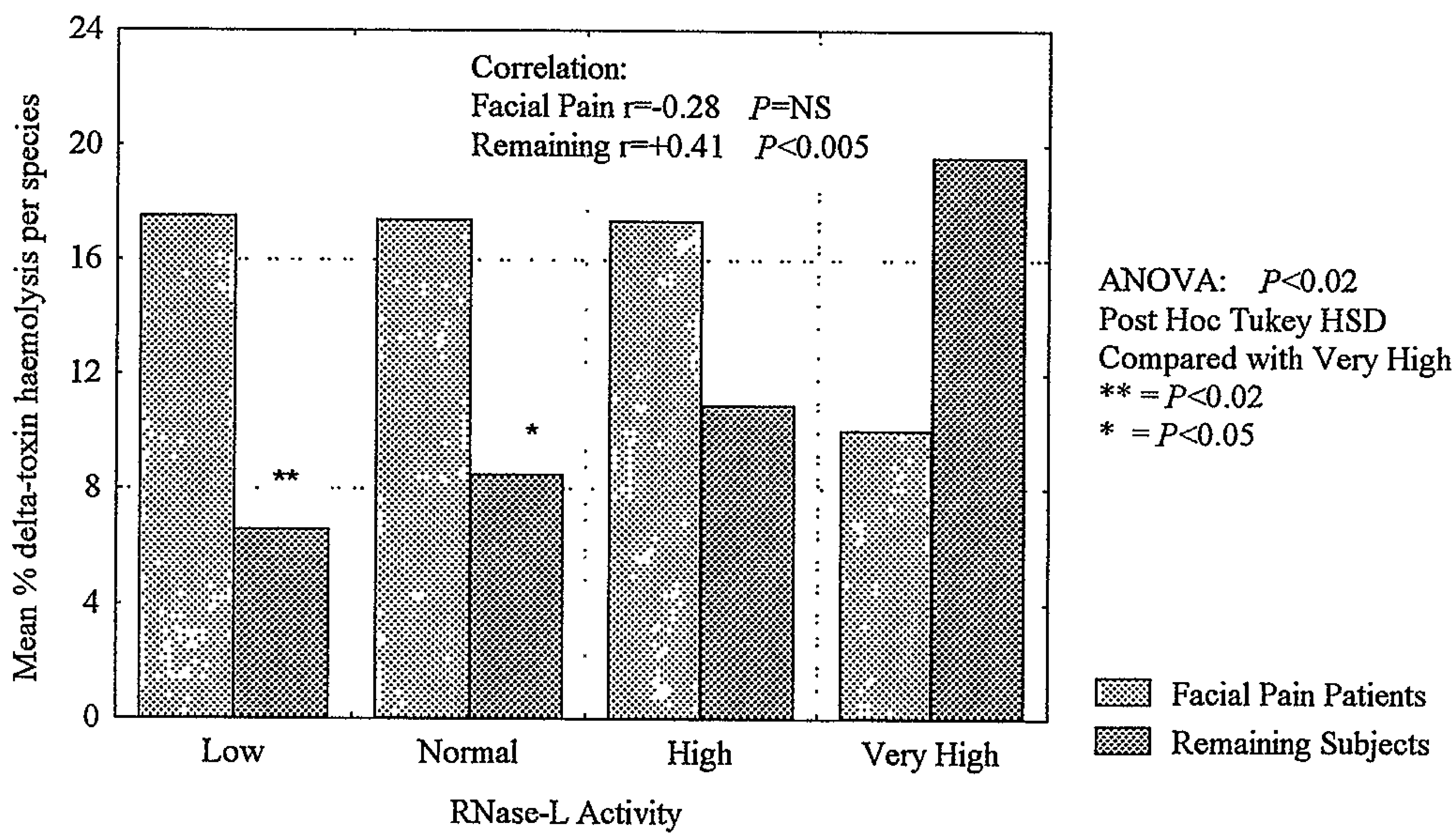
2) α-toxin (r=+0.35, $P<0.02$);

3) horse–toxin (r=+0.35, $P<0.02$) and;

4) β-toxin (r=+0.29, $P<0.05$).

In the remaining subjects IL-6 was also positively correlated with the mean percentage δ-toxin haemolysis per isolate (r=+0.34, $P<0.02$). No significant correlations were found in the facial pain patients for any of these parameters. Figure 4.6 shows the correlations between RNase-L and the mean % δ-toxin haemolysis per species in the facial pain patients and the remaining study subjects.

Figure 4.6. Association between RNase-L and the mean CoNS % delta-toxin production per species in the facial pain patients and the remaining study subjects.



Thus increases in RNase-L levels were positively associated with increases in CoNS membrane-damaging toxin production in patients who did not have facial pain. No correlations or relationships were found between any staphylococcal parameter and sIL-2r or IL-6 in any comparison.

4.4.2.f. Association between Staphylococcal Parameters and Symptoms.

Table 4.14 shows the correlation analysis between the 4 different membrane damaging toxin-producing CoNS with the scalar symptom responses. Table 4.14 also shows the same analyses weighted for the RNase-L level, which was assessed to examine the potential inter-relationship between RNase-L and CoNS toxin production. Facial pain was associated with the mean α-, β- and δ-toxin production by CoNS isolates. Weighting for RNase-L activity resulted in a reduction in these correlations.

Table 4.14. Correlation analysis between symptoms and the mean toxin-production per CoNS isolate as well as the correlation analyses weighted for the level of RNase-L.

Symptom	CoNS Toxin		CoNS Toxin + RNase-L	
Mean CoNS α-toxin/isolate		*P*		*P*
Arthritis	0.28	<0.01	0.33	<0.005
Aphthous ulceration	0.22	<0.04	0.34	<0.004
Blood pressure	0.22	<0.04	0.23	<0.05
Facial pain	0.27	<0.01	0.13	-
Headache	0.21	-	0.23	<0.05
Urinary urgency	-0.18	-	-0.34	<0.004
Chest pain	-0.03	-	-0.26	<0.03
Allergies	0.05	-	-0.23	<0.05
Mean CoNS β-toxin/isolate				
Arthritis	0.25	<0.02	0.12	-
Facial pain	0.24	<0.02	0.06	-
TMJ clicking/locking	0.15	-	0.28	<0.02
Allergies	-0.04	-	-0.31	<0.008
Irritable bowel	-0.21	-	-0.31	<0.008
Feeling hopeless about the future	-0.18	-	-0.29	<0.02
Feeling that others do not understand or are unsympathetic	-0.11	-	-0.27	<0.03
Mean CoNS δ-toxin/isolate				
Migraine headaches	0.28	<0.01	0.23	<0.05
Prolonged or unrefreshed sleep	0.31	<0.003	0.18	-
Facial pain	0.26	<0.02	0.08	-
Feeling low in energy or run down	0.25	<0.02	0.20	-
Trouble with blood pressure	0.24	<0.03	0.22	-
TMJ clicking/locking	0.15	-	0.25	<0.04
Irritable bowel	-0.15	-	-0.34	<0.004
Feeling that others do not understand or are unsympathetic	0.04	-	-0.33	<0.006
Allergies	-0.04	-	-0.32	<0.007
Diarrhoea	0.01	-	-0.28	<0.02
Urgency	-0.14	-	-0.27	<0.02
Sleeplessness	0.01	-	-0.25	<0.04
Life stresses	-0.07	-	-0.24	<0.04
Mean CoNS horse-toxin/isolate				
Prolonged or unrefreshed sleep	0.23	<0.04	0.10	-
Irritable bowel	-0.21	-	-0.43	<0.0001
Feeling that others do not understand or are unsympathetic	-0.04	-	-0.37	<0.002
Trembling	-0.07	-	-0.35	<0.003
Allergies	-0.11	-	-0.35	<0.003
Family stresses	-0.02	-	-0.34	<0.004
Bruxism	-0.08	-	-0.31	<0.007
Neck pain	-0.11	-	-0.27	<0.03
Arm pain	0.12	-	-0.27	<0.03
Life stresses	-0.15	-	-0.27	<0.03
Chest pain	0.03	-	-0.26	<0.03
Diarrhoea	0.01	-	-0.25	<0.03
Hopeless about the future	-0.01	-	-0.25	<0.04
Shoulder pain	-0.12	-	-0.25	<0.04
Feeling blue	0.08	-	-0.24	<0.05
Urinary frequency	-0.04	-	-0.23	<0.05

TMJ clicking/locking pain was not associated with any CoNS toxin levels but when weighted for RNase-L activity TMJ clicking/locking was positively associated with the mean β- and δ-toxin production per CoNS isolate. Also of importance was the association between blood pressure problems and the mean α- and δ-toxin production by CoNS isolates. Aphthous ulceration, suggested to be a reactivated viral infection associated with HHV-6 (Ghodratnama et al, 1997), was positively correlated with the mean α-toxin production by CoNS isolates and had a much higher correlation when weighted for RNase-L. Thus membrane-damaging toxin production by CoNS were positively associated with TMD symptoms, unrefreshed and prolonged sleep and alterations in blood pressure whilst the interaction with RNase-L was associated with symptom variation, particularly arthritis, aphthous ulceration and TMJ clicking/locking.

4.4.2.g. Association between Staphylococcal Parameters and Stress Responses.

Stress responses were assessed using 4 questions within the CPRU questionnaire; In the last 7-days how much were you affected by: Q35 stress from financial problems; Q79 stressful events in your life related to your problems; Q81 stress over family problems; and Q85 stress from work problems. No relationship (prevalence or severity) was found between any of these CPRU question responses and any staphylococcal parameter.

4.5. Discussion.

This study has identified an important association between the carriage of membrane-damaging toxin-producing CoNS and:

1) RDC\TMD type 1a pain symptoms;
2) pain severity in RDC\TMD type 1a pain patients; and
3) metabolic changes associated with RDC\TMD type 1a pain.

Whilst the carriage of different staphylococcal isolates per subject between the RDC\TMD type 1a pain patients and the control subjects was similar, RDC\TMD type 1a pain patients had an increase in the carriage of CoNS and toxin-producing CoNS that produce predominately "horse"- and δ-membrane-damaging haemolysins. The control subjects had higher carriage rates of CoPS compared with the RDC\TMD type 1a pain patients. Multiple regression and correlation analyses associated the carriage of toxin-producing CoNS species with increased facial muscle pain and pain severity as assessed with a VAS. An odds ratio ≥6.5 was found for the carriage of combined δ- and horse-toxin-producing CoNS and RDC\TMD type 1a pain and this was seen in patients selected on the basis of the presence of RDC\TMD type 1a pain (OR=9.2, $P<0.0004$) and in CFS patients who reported facial muscle pain (OR=6.5, $P<0.04$). The odds ratio for carriage of toxin-producing CoNS when comparing the control subjects who reported no musculoskeletal symptoms (asymptomatic control group; n=8) with the RDC\TMD type 1a pain patients was 21.7 (χ^2=10.473, P<0.002). The same association between jaw muscle pain and toxin-producing CoNS was seen in the CFS patients who reported facial muscle pain. Whilst there was no difference in odds ratios for carriage of any *Staphylococcus spp.* between the CFS patients and controls, or the CFS patients with jaw muscle pain, those CFS patients reporting jaw muscle pain had increased odds ratios for carriage of toxin-producing CoNS. It can be concluded that there is an alteration in the distribution of the staphylococcal population in the "commensal" flora of the RDC\TMD type 1a pain patients. These changes in the bacterial flora reflect a shift towards an increased colonisation of toxin producing organisms in the RDC\TMD type 1a pain group irrespective for whether they were selected on the basis of defined CFS or by the RDC\TMD criteria. No association was found between TMJ clicking/locking and any staphylococcal parameter. These data are therefore consistent with the conclusions of Plesh et al (1996), the symptom analysis in Chapter 2 and the biochemical analysis in Chapter 3, that RDC/TMD type 1a is a distinct clinical entity. This chapter associated the distinct clinical entity with a specific pathogen.

Pain severity, illness duration, body pain distribution and symptom prevalence were all positively correlated with CoNS toxin-production. The most prominent correlate for all parameters was the increase in the number of toxin-producing CoNS species per subject. Pain severity, illness duration and the body pain distribution were all negatively correlated with the number of CoPS per subject. The pattern of pain distribution associated with the toxin producing CoNS, largely resembled that described for myofascial pain syndrome (face, head, neck, arm and low back). However some of the variation in body pain distribution within myofascial pain patients may be explained by the variation in CoNS species. *Staph.epidermidis* was strongly associated with pain in the face, head and neck muscles, *Staph.lugdunensis* was associated with pain in low back, arm, leg and abdomen, *Staph.xylosis* was associated with pain in head, neck, arm and low back whilst *Staph.hominis* was associated with TMJ pain. Thus variation in the different species of CoNS was associated with variation in body pain distribution, whilst multiple carriage of toxin-producing CoNS is associated with increased body distribution of pain. In Chapter 3, variation in body pain distribution was also associated with differences in biochemistry, RNase-L, soluble IL-2 receptor and IL-6 levels. Interestingly, different strains of staphylococcus have been shown to induce different excretion of IL-1β and TNF-α (Visser et al, 1997). Thus variation in CoNS species and toxin-production and the hosts response to those pathogen-associated factors may be responsible for the differences in body pain distribution.

Although there were no strong associations between CoNS and age, Figure 4.3 shows that the age of greatest pain in RDC/TMD type 1a patients was associated with the greatest proportion of CoNS in the "commensal" flora. The peak incidence of TMD occurred at 30-40 years of age and was also the age of the highest CoNS colony count. These data show that variation in the staphylococcal flora reflects the variation in age distribution of TMD pain. The late teens and early twenties is the time when young healthy females most often develop TMD. This coincides with the period of increased staphylococcal urinary tract infections in sexually active healthy females, suggesting that contact transmission of toxin producing CoNS may be a method of spread of the TMD associated staphylococci. The association between toxin producing CoNS and TMD is strengthened by the observation that patients with RDC/TMD type 1a pain report an increased prevalence of reporting muscle pain in their long-term sexual partners, suggesting a transmissible pathogen. Whilst there was no difference in the carriage of total *Staphylococcus spp*. or CoNS species per subject, by those reporting pain in their partners, they had increased odds ratio of carriage of toxin-producing CoNS (OR=5.8, $P<0.03$) and an increase in the number of toxin-producing CoNS species per subject. The RDC/TMD type 1a patients who reported that their partners had pain, also had a reduction in the number of CoPS and the number of toxin-producing CoPS. Thus, the RDC\TMD type 1a patients who reported that their long-term partners had pain also had increased carriage of toxin-producing CoNS. These data supports the possibility of transmission of toxin-producing CoNS between partners, which may coincide with the increase in TMD symptoms in young healthy females. These observations are consistent with those of Miller et al (1996) who showed that patients on long-term antibiotics for acne and their close contacts, both carry CoNS with increased antibiotic resistance.

In the 19th century, Koch postulated that a pathogen needs to meet four fundamental requirements to comply with the term infectious pathogen. These four premises are as follows: The organism:

1. needs to be isolated from a host suffering from the disease;
2. can be cultured in the laboratory;
3. causes the same disease when introduced into another host; and
4. can be re-isolated from that second infected host.

If these requirements are satisfied, the organism is confirmed as the cause of the disease, and the disease is an infectious disease. The most criticised premise is the third, as it implies that the second host must have a genetic make-up that allows it to react to the infectious organism in the same way as the original host. This is not true for all infectious diseases. Similarly, certain pathogens may not be known or cannot be cultured in a laboratory and therefore cannot comply with Koch's postulates. The toxin-producing CoNS can be isolated from the RDC\TMD type 1a patients and cultured in the laboratory. There is evidence that the RDC\TMD type 1a patients who carry the toxin-producing CoNS have an increased incidence of partners who have pain. Pilot study data has shown that partners carry similar toxin-producing CoNS species with similar levels of toxin production, and Miller et al (1996) have shown that CoNS are transmitted by sexual contact. These data show that whilst not all Koch's postulates are complied with, the evidence supports toxin-producing CoNS as infectious agents that cause RDC/TMD type 1a pain.

Further support for an association between staphylococcal toxin-production and jaw muscle pain was evident. There was a significant relationship between jaw muscle pain and antibiotic use at onset in the RDC/TMD type 1a study group, and in multiple and prolonged antibiotic use by the CFS study patients as reported in Chapter 2. In the RDC/TMD type 1a group, the use of antibiotics at onset was associated with higher CoNS colony counts; and increased mean and maximum δ-toxin production rates, compared with those RDC/TMD type 1a patients who did not report antibiotic use at onset. Similarly those RDC/TMD type 1a pain patients reporting antibiotic use at onset had a higher odds ratio for carriage of toxin-producing CoNS (OR>100, $P<0.0006$), compared with control subjects as well as the RDC/TMD type 1a pain patients not reporting antibiotic use at onset (OR=13.8, $P<0.002$). Thus antibiotic use at onset of the illness was associated with an increase in the δ-toxin-producing CoNS isolated from the RDC/TMD type 1a patients. This is consistent with the selection of higher toxin-producing CoNS by antibiotics. Survival of CoNS would be associated with upregulation of the *arg* gene related production of antibiotic defence proteins such as penicillin binding protein (Kernodle et al, 1995; Piriz Duran et al, 1996). Kernodle et al, (1995) showed that sub-lethal doses of penicillin resulted in higher toxin production by both *arg* and *arg*-independent mechanisms. These data are consistent with the increased levels of antibiotic resistant CoNS strains following antibiotic use (Miller et al, 1996). In this study the post-antibiotic CoNS had increased levels of δ-toxin production, which is part of the *arg* gene system (Recsei et al, 1986; Arvand et al, 1990; Janzon & Arvidson, 1990; Regassa et al, 1992), suggesting increased *arg* gene products are produced by those CoNS carried by the RDC/TMD type 1a patients. Thus, antibiotic use may lead to the selection of greater toxin-producing CoNS, which in turn may lead to the development of muscle pain.

Stressful life experiences have been claimed to be associated with onset of TMD. However in Chapter 2 and 3, no strong relationship was found between stressful events and TMD symptom expression, and the biochemical changes associated with jaw muscle pain. In this chapter, no association was found between stress and any staphylococcal parameter. Similarly increased SCL-90-R depression scores are suggested to be associated with TMD, however the data indicates that there was no significant relationship between SCL-90-R depression dimension scores and TMD symptom expression (Chapter 2), biochemical changes (Chapter 3) or any staphylococcal parameter. Thus no significant relationship was found between jaw muscle pain and depression or stress.

Seventy percent of TMD patients report a gradual onset of their condition that progresses from a localised pain condition to a more widespread pain distribution. In chapter 2 and 3 this gradual onset was found to be associated with a progressive loss of metabolite by what appeared to be a nitric oxide mediated diuresis. Patients reporting a gradual onset, had a higher odds ratio for carriage of toxin-producing CoNS (OR>100, $P<0.00004$) than those reporting a sudden onset (OR=2.92, $P<0.02$), when both groups were compared with the control group. However, no difference was found when comparing the sudden and gradual onset groups. Importantly, the illness duration was positively correlated with increases in carriage of toxin-producing CoNS. These data suggest that toxin-producing CoNS are associated with biochemical changes and the progressive loss of metabolite.

The CFS patients in the RNase-L study group had increased maximum percentage haemolysis per subject and mean percentage haemolysis per isolate for CoNS β- and δ-toxins. Whilst no association was found between RNase-L, soluble IL-2 receptor and IL-6 levels and any staphylococcal parameter, when all study subjects were included. There was a significant association found between RNase-L and CoNS δ-toxin in the study subjects who did not report jaw muscle pain. In the non-facial pain subjects positive correlations were found between RNase-L and the maximum percentage δ-toxin per subject, and the mean percentage haemolysis per isolate for all four CoNS membrane damaging toxins (α,β, δ and horse). IL-6 levels were also positively correlated with the mean percentage δ-toxin haemolysis per isolate in the non-facial pain patients. These data have several important implications. Facial pain patients have a higher carriage rate of toxin-producing CoNS than other subjects, which is independent of RNase-L, IL-6 and sIL-2r, and is consistent with RDC/TMD type 1a pain being a distinct clinical entity. RNase-L, an indicator of increased IFN associated antiviral activity, was positively associated with increases in CoNS toxin-production in patients who do not report jaw muscle pain. CFS patients have an increased prevalence of RDC/TMD type 1a pain (Chapter 2) and their condition has been linked to reactivation of a number of different viruses (Bowles et al, 1993; Manian 1994; Yalcin et al, 1994; Di Luca et al, 1995; Sairenji et al, 1995), particularly HHV-6 (Buchwald et al, 1992; Suhadolnik et al, 1994; Patnaik et al, 1995). There is no evidence of new aetiologically related viral infections in CFS patients. It is likely that an interaction between two or more pathogens (viruses and/or bacteria) and an environmental or genetic factor, is one mechanism that results in reactivation of viruses in CFS patients. Clementsen et al (1990a) also showed that viruses, such as Influenza A virus, can enhance basophil histamine release induced by *Staphylococcus aureus*, which when associated with an allergen can induce an exaggerated histamine response in asthmatic patients (Oehling et al, 1997). In this chapter increases in α-toxin and RNase-L were positively associated with recurrent aphthous ulceration, suggesting that cell permeabilization by channel-forming CoNS toxins was associated with increases in RNase-L and hence viral reactivation. It appears that a combination of viral infections and staphylococcus toxins were associated with exaggerated symptom responses, including TMD symptom expression and alterations in blood pressure. These events may explain the increased prevalence of TMD symptoms in CFS (Chapter 2) and fibromyalgia patients (Plesh et al, 1996), as combined pathogen associated events may potentiate each other and result in an increase in patient morbidity.

This study revealed that α- and δ-toxin production per CoNS isolate was associated with 7-day facial pain and problems with blood pressure in the CFS study group. These symptoms are consistent with the biochemical findings in chapter 3 where the level of sodium was found to be the major factor associated with 7-day facial pain expression and that the loss of metabolite. In the RDC/TMD type 1a patients the increase in toxin-producing CoNS in the RDC/TMD type 1a patients was positively associated with unknown UM28, the tyrosine: leucine ratio, the excitatory amino acids, glutamic acid and a series unknown urinary metabolites: UM15a, UM13a, UM27 and UM13. These data indicate a positive association between increases in the urinary excretion of excitatory amino acids, and a series of unknown metabolites and CoNS toxicity in the RDC\TMD type 1a pain patients. The urinary tyrosine: leucine ratio, an indicator of a ratio of proteolysis to protein synthesis, was elevated in the RDC\TMD type 1a pain patients. The increase in the tyrosine: leucine ratio and the reduction in leucine with increasing carriage of toxin producing CoNS are consistent with an association between staphylococcal membrane-damaging toxin production and dysregulated proteolysis. Collectively, these data implicate CoNS toxicity in the development of the biochemical changes associated with TMD type 1a pain expression. The association between bacterial toxicity and dysregulated protein metabolism may also occur via an IFN-γ associated process that may result in a similar clinical outcome to that seen with viral induction of IFN-γ. The unknown urinary marker, UM28, noted in the RDC\TMD type 1a pain patients was strongly associated with the carriage incidence of toxin producing CoNS and may prove to be a valuable diagnostic marker in the study of patients with chronic muscle pain. The fact that symptom-free control subjects in this study did not carry toxin producing CoNS, further suggest that coagulase-negative staphylococcal membrane-damaging toxins are associated with dysregulation of protein turnover and pain expression in TMD patients.

Toxin producing CoNS were positively associated with pain severity as well as increases in the urinary excretion of the excitatory amino acid, glutamic acid in RDC\TMD type 1a pain patients. Coderre (1993) proposed that increases in the tissue and spinal fluid concentrations of excitatory amino acids are required in the activation of spinal cord N-methyl-D-aspartate (NMDA) receptors, which are associated with the induction of hyperalgesia. Coderre (1993) also proposed that increased sensitisation of both peripheral nociceptors and the central nervous system can be attributed to increases in the turnover of glutamic and aspartic acids. The continual presence of a skin or mucosal membrane lipid-soluble toxin producing staphylococcus is likely to produce a persistent stimulus for the chronic pain response. The lipid solubility of the toxins ensures that they may cross the epithelial barrier without the necessity for an infection to induce a toxic reaction. Therefore toxin producing CoNS comply with most of the requirements specified by Koch. These are a persistence stimulus, increased excitatory amino acid availability and a toxic stimulus that initiates an alteration in protein turnover and nitric oxide responses, which appear necessary for the development of chronic muscle pain.

One of the aims of this chapter was to assess the role of toxin-producing CoNS in the expression of TMD. The CoNS toxins were found to be associated with jaw muscle pain expression in patients selected on the basis of having either RDC/TMD type 1a pain or Chronic Fatigue Syndrome. This suggests that jaw muscle pain has a distinct clinic entity and that the CoNS toxins are associated with its aetiology. The toxicity associated with variation in the hosts' immune system and the other pathogens that have infected the host may influence the morbidity of other conditions, such as CFS. In fact CoNS toxins may be agents that are capable of inducing reactivation of viruses, as evidenced by the association between

RNase-L, CoNS α-toxin and the severity of aphthous ulceration. Thus, toxin producing CoNS are capable of inducing a change in the host that is consistent with a nitric oxide mediated immune response and therefore fulfils the requirements to be classified as a human pathogen.

4.6. Conclusion

These data present a new hypothesis for the aetiology of chronic muscle pain disorders. Patients colonised with lipid soluble membrane-damaging toxin-producing coagulase-negative staphylococci, are associated with chronic muscle pain disorders. *Staphylococcus spp.* are generally considered part of the normal commensal flora and of little pathological significance. This study suggests that membrane-damaging toxin producing staphylococci, should no longer be viewed as non-pathogenic. Furthermore, it is proposed that future management will require measurement and neutralisation or suppression of specific toxin-producing staphylococci as a relevant diagnostic tool and treatment approach for chronic myofascial pain patients.

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CHAPTER 5.

5. Conclusions: Facial Muscle Pain: an Hypothesis and Future Research.

5.1. Overview.

Chronic muscle pain conditions form a group of syndromes that have many similar characteristics. These heterogeneous yet overlapping groups of conditions may intermittently affect up to 70% of the population with severe forms, such as fibromyalgia, occurring between 5-10%. These conditions represent very common clinical and therapeutic problems with unknown aetiology. A number of hypotheses have been proposed to explain the complex aetiology of TMD disorders (reviewed in Chapter 1). However this study has found a significant set of biochemical and microbiological associations which allow the development of a novel model for the aetiopathogenesis of defined RDC/TMD type 1a muscle pain (Le Resche et al, 1992). **These data also allow the description of a new disease entity: coagulase-negative staphylococcal toxaemia**.

Chapter 1 outlined pilot study data from 35 chronic orofacial muscle pain patients, where an association was found with multi-organ symptoms and a history of urinary tract infection, altered urinary sodium levels and increased staphylococcal counts (McGregor et al, 1992; 1993a; 1993b). As a result, studies were commenced to assess the association between TMD symptoms and pain characteristics, Hopkins Symptom Checklist-90-Revised (SCL-90-R) responses, microbiology, whole body symptom prevalence and onset events. These measures were assessed using 3 separate studies: 1) analysis of defined RDC/TMD type 1a patients; 2) TMD expression in defined CFS patients (Royal North Shore Hospital Collaborative study); and 3) a study by Ms Lee Metcalf of staphylococcal toxin-production in CFS patients and control subjects. These three studies have allowed differentiation of the changes in biochemistry and staphylococcal toxicity associated with TMD symptoms. The data analysed in this thesis confirmed the pilot study data and allowed the development of a model to describe the aetiopathogenesis of RDC\TDM type 1a muscle pain or myofascial pain.

5.2. Coagulase-negative Staphylococcal Toxaemia.

As the available data show a strong compliance with Koch's postulates (Chapter 4 page 211-212) it is proposed that coagulase-negative staphylococcal toxaemia be defined as a separate disease entity. Coagulase-negative staphylococcal toxaemia is a disease induced by toxicogenic coagulase negative staphylococcus species and is diagnosed by the isolation of membrane damaging toxin-producing coagulase negative staphylococci in patients reporting a symptom constellation consistent with myofascial pain syndrome. These bacteria are most likely the major pathogen, although are not the only pathogen involved in development of chronic muscle pain. The carriage of these organisms is associated with the symptoms summarised in Table 5.1.

Table 5.1. Symptoms associated with coagulase-negative staphylococcal toxaemia (from Tables 4.9 and 4.14).

Symptom
Musculoskeletal symptoms
Jaw muscle pain
TMJ clicking or locking
Neck/Shoulder pain or tenderness
Arm pain or tenderness
Sciatica
Arthritis/Painful or stiff joints
Infectious symptoms
Swollen or tender cervical and/or axilliary lymph nodes
Night sweats
Sore throat
Recurrent sinusitis
Aphthous ulceration
Other symptoms
Hyperaesthesia/Paraesthesia
Clenching or grinding of the teeth
Earaches and Tinnitus
Headache and Migraine headaches
Hair loss
Irritable bowel and abdominal symptoms
Diarrhoea
Palpitations
Blood pressure problems
Faintness / dizziness
Muscle fatigue
Generalised lethargy and sleep disturbance

Most patients report a gradual onset with symptoms first reported in the jaw/neck/shoulder (fascioscapulohumeral) muscles and in those patients reporting a definitive onset, many report a sore throat at onset. The condition can be acquired by bacterial transfer within a family, by close contact with a partner or can develop after prolonged use of antibiotics that select for more toxicogenic staphylococcal species. The condition can increase the morbidity of other conditions such as CFS and fibromyalgia and may potentiate symptom expression in patients with other immune activated states with increased cytokine production, such as HIV and carcinomas. Onset is likely to be gradual in about 70% of cases and most female patients are likely to have lower normal blood pressures and to report an increased frequency of urination during periods of symptom exacerbation. Thirty percent of subjects will report a sudden onset and are likely to develop an increase in blood pressure due to the increase in serum tyrosine availability and then to have continued erratic labile blood pressures. The patients who develop jaw muscle pain differed from the remaining toxaemic patients as they have lower blood pressures, serum sodium and tyrosine levels than those with labile blood pressures. This difference appears to be associated with the ability to maintain normal sodium and catecholamine levels.

Treatment is currently a significant problem as these organisms are primary colonisers of skin and mucosal surfaces and antibiotic usage is unlikely to remove the organisms for a prolonged time, and in fact may select for more toxicogenic species. The toxins are of very low molecular weight (26 amino acids or less) and consequently have low antigenicity. Hence, patients are unlikely to develop a normal immune response against these toxins. The development of better treatment strategies such as vaccination against the toxins is required. Murphy (1935) reported that patients with toxin-producing staphylococcal problems had "general lassitude, inability to concentrate, and ready fatiguability being common complaints. Sleep was liable to be troubled and unrefreshed. ...others had multiple rheumatic pains." Murphy reported that following the use of toxoid vaccination "Despite the clinical improvement, the bacteriological findings were in general unchanged. The definite early benefit resulting from toxoid immunisation in this type of case cannot be ascribed to elimination of the staphylococcus, but presumably derives from the increased titre of circulating staphylococcus antitoxin." Thus, the reduction in toxaemia was associated with the reduction in symptom expression. These vaccines have been removed from the market apart from the "Staphypen" vaccine[✝]. The development of vaccines or competitive proteins against the cyclic octapeptide pheromone (Balaban & Novick, 1995; Ji et al, 1995) need to be evaluated as these may reduce toxin production by the coagulase-negative staphylococci.

5.3. A Model of the Aetiopathogenesis of RDC/TMD Type 1a Muscle Pain.

The novel model for the aetiopathogenesis of RDC\TMD type 1a muscle pain or myofascial pain syndrome will be discussed in 3 sections;

1. The mechanisms of muscle pain development;
2. Sex differences; and
3. Treatment approaches.

[✝] "Staphypen", Swiss vaccine institute of Berne, Switzerland.

5.3.1. The mechanism of muscle pain development.

5.3.1.a. Spinal hyperalgesia.

The acute pain spinal hyperalgesia model developed from research as reviewed by Coderre (1993) and Coderre & Yashpal (1994) is show in Figure 5.1. This model is not applicable to the chronic pain model but can be modified to reflect the changes noted in the series of studies reported in this thesis.

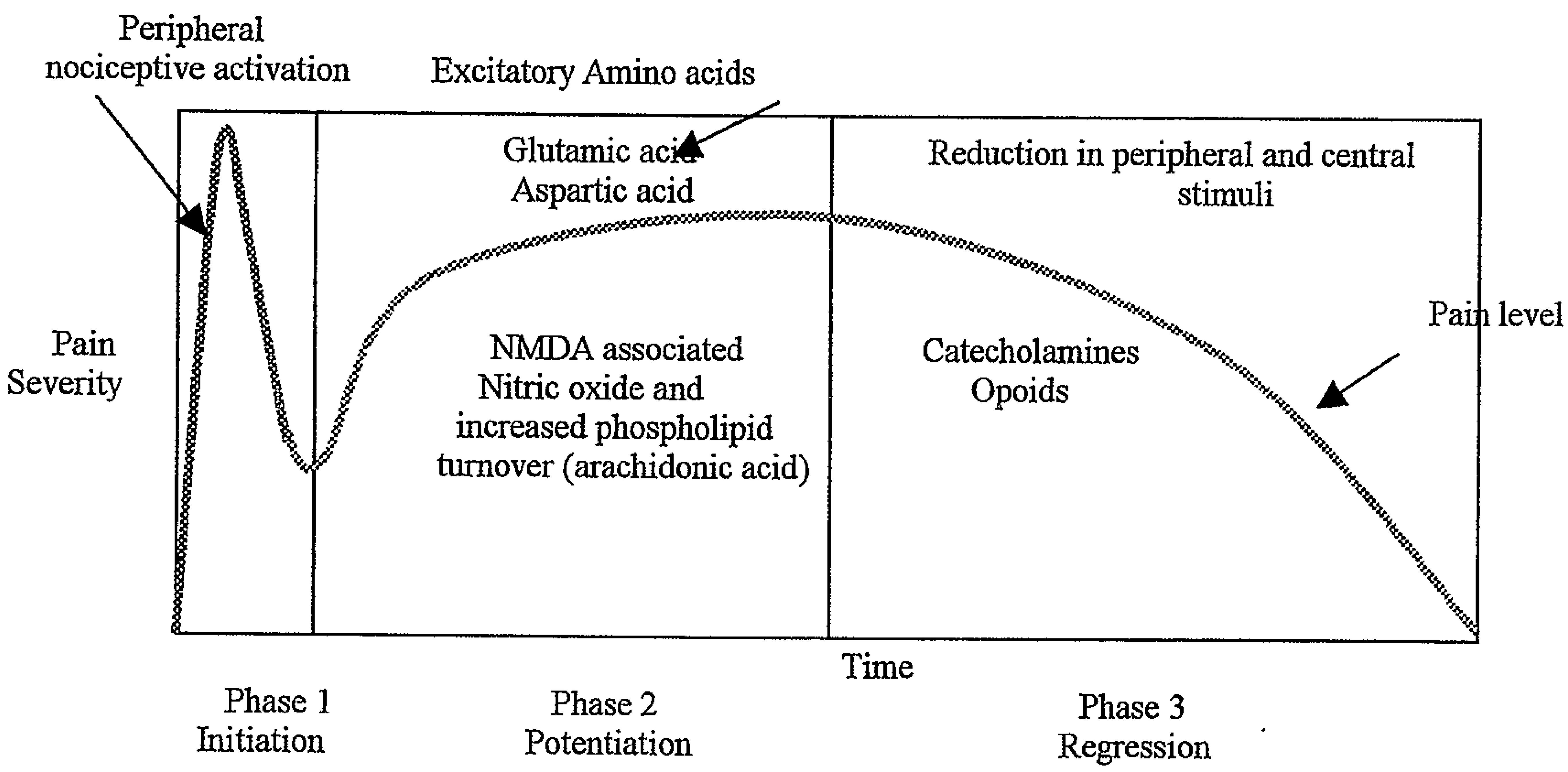

Figure 5.1. Summary of the phases of the hyperalgesia pain response in the spinal cord related to pain severity.

Phase 1. Peripheral nociceptive activation of the pain response. e.g. trauma to tissue.

Phase 2. Potentiation of hyperalgesic response in the spinal column. Increased excitatory amino acid levels activate the NMDA receptor and initiate a nitric oxide mediated response. $Na^{+}K^{+}$ATPase activity is inhibited (Tavalin et al, 1997; Sato et al, 1997) and the hyperalgesic response is initiated.

Phase 3. A reduction in the peripheral stimuli and potentiation phase components along with increases in catecholamines and opoids inhibit the hyperalgesic response.

Figure 5.2 shows the model for the development of hyperalgesia in myofascial pain patients developed from the data revealed in the studies presented in this thesis. The myofascial pain hyperalgesia response lacks the initiating peripheral nociceptive stimulus seen in the acute pain model and is replaced by a series of biochemical events that lead to upregulation of what was phase 2 of the acute model. Therefore, the model consists of two phases: Phase 1) an initiation/potentiation phase; Phase 2) a resolution phase. Phase 1 is associated with increased coagulase-negative staphylococcus (CoNS) species membrane damaging toxin production, particularly δ- and horse-toxin. Increases in CoNS toxin production were associated with increases in jaw muscle pain and alterations in blood pressure, increases in excretion of the excitatory amino acid, glutamic acid, and the tyrosine: leucine ratio. Jaw muscle pain was associated with increases in the excretion of the excitatory amino acids, glutamic and aspartic acid, the phospholipid polar head-group amino acids, ethanolamine and serine, and evidence of nitric oxide mediated alteration in kidney function (reduction in serum sodium, increased urine volume and total metabolite excreted).

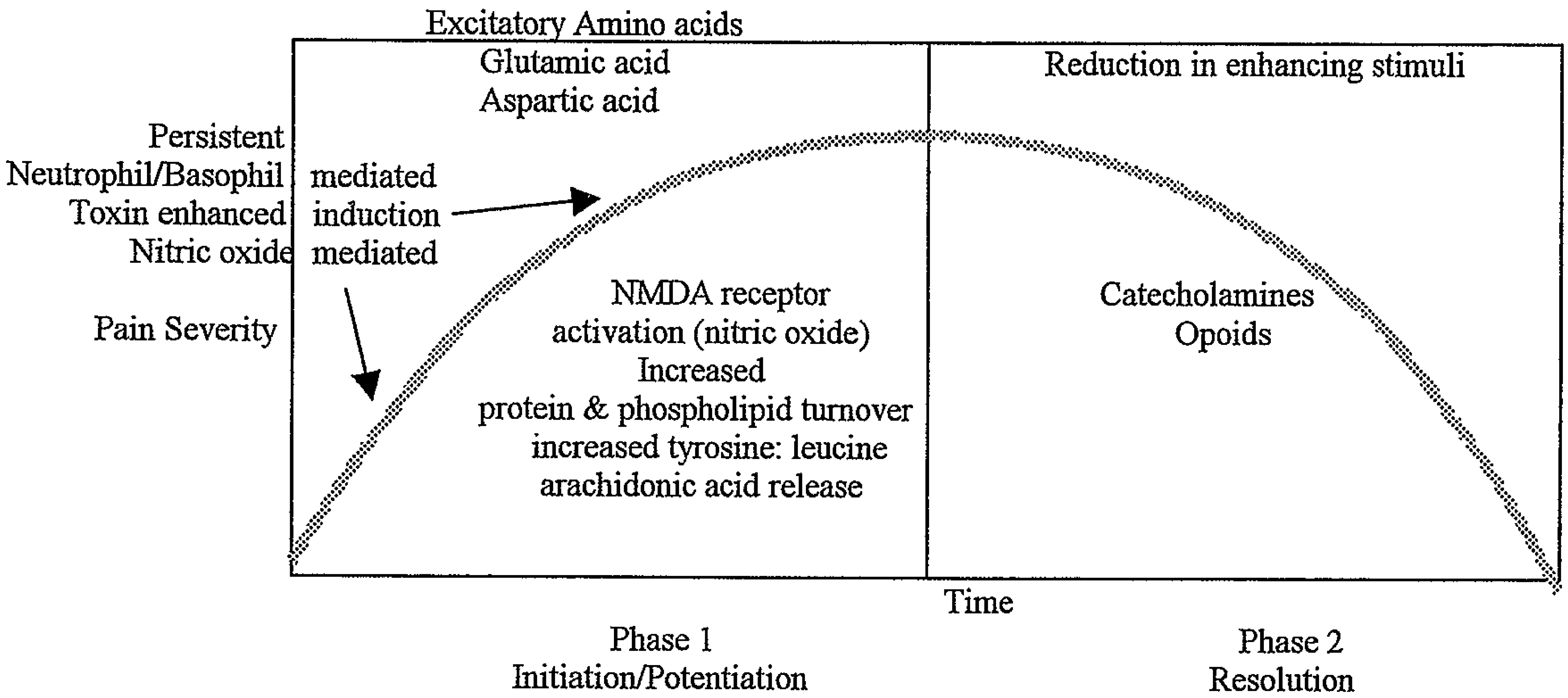

Figure 5.2. Summary of the proposed phases of the muscle hyperalgesia pain response.

Phase 1. Cytokine/toxin mediated/potentiated activation of nitric oxide response with initiation and potentiation of the hyperalgesic response in the spinal column. The same biochemical changes initiate diuresis, low-grade aminoaciduria and sodium loss from the kidney (Haynes et al, 1997) and an alteration in the hypothalamic pituitary adrenal and gonadal axes. Increased excitatory amino acid levels activate the NMDA receptor and initiate a nitric oxide mediated response within the spinal column. Increased Phospholipase activity and release of lipid associated pain mediators, such as arachidonic acid, facilitate increases in prostaglandins and leukotrienes. $Na^{+}K^{+}$ATPase activity is inhibited and the hyperalgesic response is initiated as a result of alteration in membrane potential (a nitric oxide-mediated $Na^{+}K^{+}$ATPase channelopathy-like situation). The reduction in serum tyrosine will result in a reduction in catecholamines and failure to inhibit the pain response and a reduction of dopamine that will facilitate the kidney diuresis and loss of electrolyte and amino acids. Progressive loss of amino and organic acid components in urine result in additional loss of neurotransmitter precursors and dysregulation of cognitive and neurological functions that prevent the initiation of the resolution phase.

Phase 2. A reduction in the cytokine/toxin stimuli and potentiation phase components along with restoration in amino and organic acid loss and increases in catecholamines and opoids inhibit the hyperalgesic response.

These data strongly support the changes proposed by Coderre & Yashpal (1994) for the phase two potentiation component of the acute pain response. Thus it is proposed (see Figure 5.2) that the initiation of the hyperaesthesia component in chronic jaw muscle pain is established by increases in CoNS δ- and horse- membrane damaging toxins and the body's inflammatory-mediated response to these toxins (dysregulated immune response, increased basophil count). These events result in a NMDA/nitric oxide mediated event. The increased nitric oxide response results in a diuresis event associated with loss of sodium, amino and organic acids, and an alteration in the hypothalamic pituitary adrenal/gonadal axes. The inflammatory mediated increase in phospholipid turnover would be associated with the same type of responses noted during phase 2 of the acute hyperalgesic response but due to the persistence of the stimuli would be reduced in severity. The increased phospholipid turnover was also found to be associated with increased accumulation of pesticides, DDE and deildrin. The accumulation of these secondary substances results in increased symptom expression and alterations in biochemistry consistent with dysregulation of glucose transport and GABA receptor activity within the brain stem.

5.3.1.b. Kidney Diuresis.

As the hyperalgesia response is associated with an increase in nitric oxide and a reduction in catecholamine, kidney function will also be altered. The excretion of sodium, fluid and metabolites from the kidney are regulated by a series of hormones (e.g. Adrenocorticotrophic hormone; ACTH), however the actions of these hormones are facilitated by the local production of prostaglandins, nitric oxide and dopamine. An increase in pain and body pain distribution is associated with an increase in urine volume, total urine metabolites and a reduction in serum catecholamine precursors, tyrosine and phenylalanine. The increase in urine volume was correlated with a reduction in serum tyrosine levels. Thus an increase in nitric oxide production or a reduction in dopamine levels is associated with an increase in loss of fluid, sodium and metabolites from the kidney and this correlates with pain severity.

Although not measured in the studies described in this thesis, the levels of various fatty acids which result in an increase in prostaglandin E_2 production will effect blood pressure and renal handling of sodium. The pro-inflammatory fatty acids from the n-6 grouping (linoleic acid, arachidonic acid) which are involved in the pain process (Coderre & Yashpal, 1994), are also involved in regulation of renal function (Iacono & Dougherty, 1993). Thus not only alterations in nitric oxide and dopamine but also prostaglandin precursor fatty acids may also be involved in the renal diuresis noted with pain. Analysis of the pilot data from the Royal North Shore Hospital study shows that urine volume is positively correlated with the total n-6 fatty acid levels in the CFS patients group (n=15; r=0.59, $P<0.02$). It appears that: a) the biochemical changes associated with pain severity are essentially the same as those that induce renal diuresis; and b) the biochemical variables associated with increasing pain severity, influence metabolism in the kidney as well as the spinal column.

5.3.1.c. Hypothalamic-Pituitary Adrenal Axis.

Adrenocorticotrophic hormone (ACTH) and therefore the hypothalamic-pituitary adrenal (HPA) axis may also play a role in the diuresis seen with chronic pain. Figure 5.3 shows a schematic summary of the inter-relationship between cytokine production, the HPA axis and the immune system. Alterations in the production of cytokines, such as IL-1β and IL-6, have been shown to regulate these systems (Wilder, 1995). This HPA axis is however subject to changes in nitric oxide production and the availability of histamine and the catecholamine, noradrenaline. As with the spinal hyperalgesic response and the dysregulation of kidney function, nitric oxide- and series 2 prostaglandin- mediated events are associated with activation of the HPA axis, whilst catecholamines such as noradrenaline are predominantly inhibitory in this process. Thus, dysregulation of the HPA axis and its central processing are highly likely to occur in chronic pain conditions. This has been confirmed by the studies of patients with fibromyalgia (Crofford et al, 1994) and CFS (Cleare et al, 1995).

Variations in the cytokines and the HPA axis response are likely to be involved in variation in symptom expression. In jaw muscle pain patients there were increases in basophil and neutrophil counts suggesting that increases in IL-4, histamine and a Th2 immune response may be involved in jaw muscle pain development. Hypothalamic histamine levels have been associated learning and memory (Kamei et al, 1997), thermoregulation and alterations in masticatory activity and satiation (Sakata et al, 1997). The cytokine IL-1β through activation of PGE_2 increases histamine turnover; the resultant reduction in histamine leads to hyperphagia, disruption of feeding circadian rhythm, hyperlipidemia, hyperinsulinemia, and disturbance of thermoregulation (Sakata et al, 1997). Interestingly, both Russel et al (1989) and Yunus et al (1992) showed that patients with fibromyalgia had reduced histidine levels and Yanai et al (1997) showed that excitatory amino acids that activate NMDA receptors (glutamine/aspartic acid) can induce excitotoxicity in hypothalamic histaminergic neurones. Thus, the increase in peripheral basophil counts may be a response to reduction in central histamine levels.

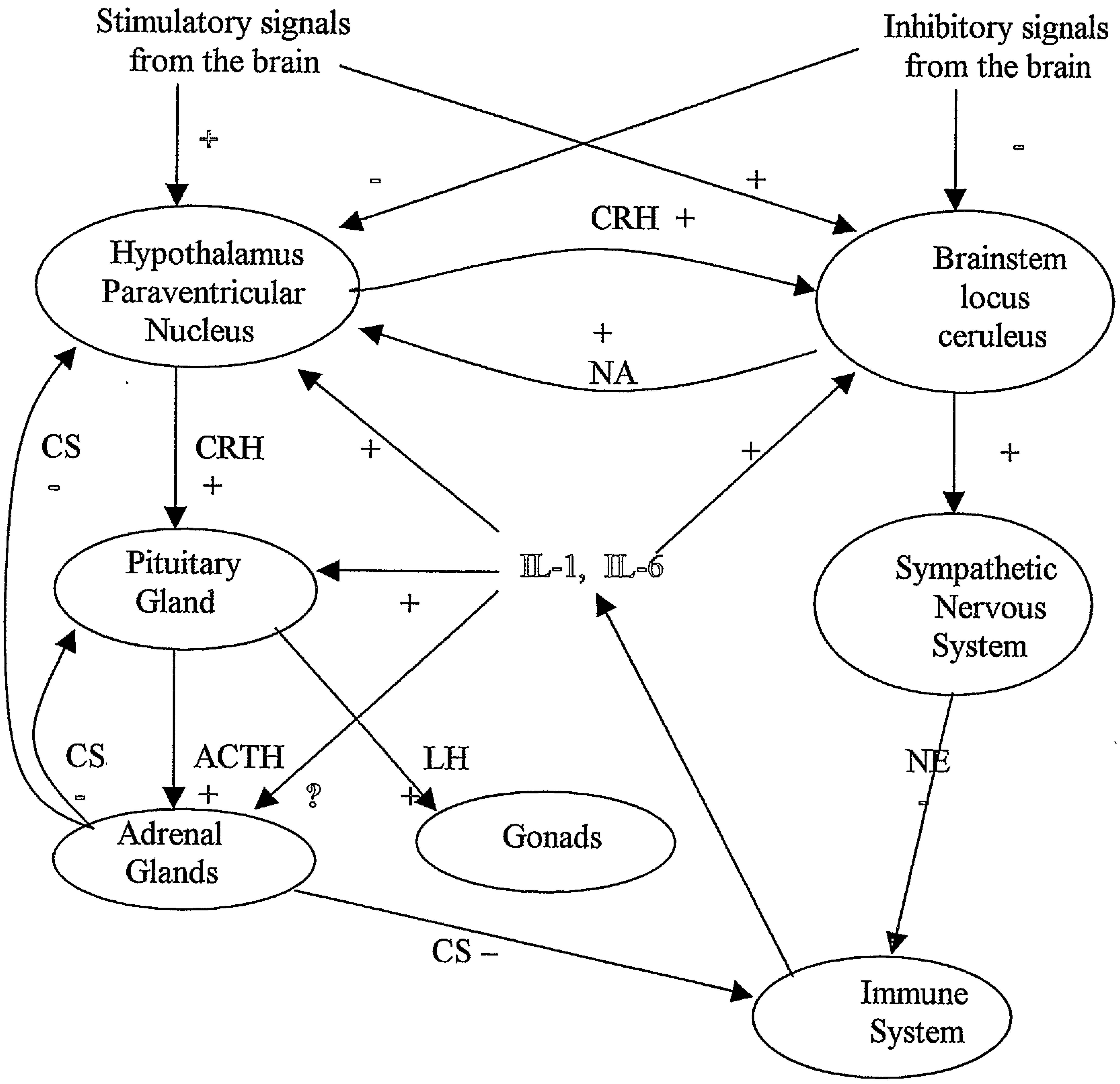

Figure 5.3. The associations between immune cell products (cytokines IL-1, IL6) and the hypothalamic pituitary adrenal axis (HPA) and the hypothalamic pituitary gonadal axis (HPG) feedback mechanisms. In chronic pain patients the increase in cytokine levels are associated with alterations in the balance of the feedback mechanisms. In the jaw muscle pain patients alterations in sodium availability, histamine, catecholamines, prostaglandins and nitric oxide will significantly alter the balance between these interactions. Each of the steps listed in the above figure are also driven by alterations in the availability of precursors. The fall in serum tyrosine and hence noradrenaline availability would significantly alter the sympathetic nervous system inhibitory control over immune function. Similarly the accumulation of Deildrin and DDE in the membranes will further dysregulate catecholamine activity and γ-aminobutyrate acid (GABA) receptors removing more of the inhibitory influences over the HPA axis. This research data strongly suggests that an increase in excitatory factors and a reduction in inhibitory factors are occurring with chronic pain and that these may be associated with dysregulation of the HPA axis. (Modified from Wilder, 1995).

ACTH = Adrenocorticotrophic hormone; CRH = Corticotrophin releasing factor; CS = Corticosteroid; LH = Luteinizing hormone; NA = Noradrenaline

In the CFS study the basophil count was negatively correlated with SCL-90-R 7-day response for Q19 (poor appetite: n=72, r=-0.25, $P<0.03$), and was positively correlated with the 12-month frequency response for SCL-90-R Q60 (overeating: n=69, r=+0.27, $P<0.03$). It appears that alterations in basophil counts are associated with dysregulation of hypothalamus-associated histamine metabolism in chronic pain patients, and with alterations in symptoms and satiation (Sakata et al, 1997).

The accumulation of organochlorine pesticides Dieldrin and DDE also appear to effect the HPA axis. Dieldrin has been shown to be toxic to both GABA-ergic and dopamin-eric neurones in the central nervous system (Sanchez-Ramos et al, 1998). Interestingly, histamine release in the anterior hypothalamus is dependent upon GABA (Okakura-Mochizuki et al, 1996). It is not surprising therefore, that the accumulation of the organochlorines with duration of the illness is associated with alterations in the HPA axis.

Experimental sepsis in rats resulted in a reduction in serum arginine and tyrosine by 24 hours and an increase in brain serotonin levels by 48 hours and was associated with a significant reduction in the ability of the rats to learn new tasks (Shimizi et al, 1999). This is consistent with the altered cognition noted in CFS and chronic muscle pain patients. Cytokine and lipopolysaccharide alterations have been shown to dysregulate nitric oxide production in different parts of the brain (Wang & Dunn, 1998; Konsman et al, 1999) and in turn are associated with alterations in tyrosine hydroxylase activity and dysregulation of hormone production such as prolactin and cortisol (Gonzalez et al, 1998).

Alterations in cytokines and hypothalamic-pituitary adrenal axis responses to the cytokine and staphylococcal toxins may play a significant role in the alterations in brain function seen in chronic pain patients. Cytokine injection have as their major side effects pain, fatigue and depressed mood (Dusheiko 1997; Allen-Mersh et al, 1998; Valentine et al, 1998). Allen-Mersh et al (1998) showed that increased soluble IL-2 receptor levels were associated with depression in patients with bowel carcinoma whilst Cleare et al, (1995) has shown that the HPA axis responses were different between patients with depression compared with CFS patients. The immune cell parameters and therefore the cytokines associated with jaw muscle pain are likely to induce their own unique HPA axis change. The responses associated with jaw muscle pain are likely to be associated with variation in toxins, histamine and neutrophil products. They may exacerbate other pain conditions or induced responses with certain patient subgroups, leading to enhanced symptom expression. These data therefore warrant specific studies examining these potential changes in RDC/TMD type 1a pain patients, and to determine the associations with staphylococcal toxins.

5.3.1.d. Immune cell changes.

Chronic pain mechanism appears to result from a reduction in inhibitory factors (catecholamines) and enhancement of stimulatory factors that alter spinal nerve hyperalgesia, HPA axis and kidney function. The factors that drive these alterations are: a) toxins from various bacteria, in the case of jaw muscle pain coagulase negative staphylococcal membrane damaging toxins; and b) the hosts cytokine and immune cell product responses. Proof of this is seen with the therapeutic injection of cytokines, which result in variable expression of pain, fatigue and altered mood (Dusheiko 1997; Allen-Mersh et al, 1998; Valentine et al, 1998). In this study increases in jaw muscle pain in the previous 7-days were associated with an increased percentage of neutrophils in the CFS patients. This suggests two major influences: a) the increase in neutrophils occurs as a result of a bacterial infection/toxicity problem; or b) dysregulation of catecholamine metabolism is associated with the increase in neutrophils. Both of these events are likely to occur and may act synergistically in RDC\TMD type 1a pain patients.

The increase in 12-month frequency and severity of jaw muscle pain was strongly associated with increases in basophil and neutrophil counts and a reduction in the lymphocyte count (12-month frequency). The increase in 12-month frequency of jaw muscle pain and pain severity were also associated with an increase in liver enzymes and markers of tissue damage, similar to that seen following severe exercise programs (Kayashima et al, 1995; Mena et al, 1996; Bruunsgaard et al, 1997). The tissue damage markers were correlated with increases in basophil and neutrophil counts. These data suggest that the immune cell products are strongly associated with the development of jaw muscle pain.

Cytokines and bacterial toxins have been shown to exacerbate each others effects *in-vitro*. In this study, when the coagulase negative staphylococcal toxin correlation analyses were weighted with the cytokine and RNase-L responses, it was noted that certain symptom sets were amplified in their correlation values over that seen by either parameter alone. Thus coagulase negative staphylococcal toxins were associated with increased symptom severity and an increase the morbidity of pain patients with other conditions such as CFS. In this research, CFS patients with myofascial pain had increased symptom prevalence compared with CFS patients who did not have myofascial pain. Similarly, Bombardier & Buchwald (1996) showed that patients with both CFS and fibromyalgia had greater disability than patients diagnosed with either condition alone (Bombardier & Buchwald, 1996; Buchwald et al, 1996). Interaction between bacterial toxins and immune cell products, together with other aetiological agents are associated with symptom variation and increased morbidity in chronic pain patients.

5.3.1.e. Gradual onset and Duration.

In the RDC/TMD type 1a pain patients, the total metabolite excreted was positively associated with increases in pain severity and pain distribution and negatively correlated with the duration of the illness. The total metabolite excreted was also higher in RDC/TMD type 1a pain patients where there was a sudden onset. In the CFS patients, the increase in jaw muscle pain was associated with an increase in urine volume, but the CFS patients had a reduced urine volume. These data show that a progressive loss of metabolite and fluid is occurring with myofascial pain. In 60% of cases of myofascial pain (Weinberg & Lager, 1980; Fricton et al, 1985; Bengtsson et al, 1986) the patients report a gradual onset. Most chronic RDC/TMD type 1a pain patients reported a localised pain pattern at onset that progressed to a more wide-spread pain distribution. If a repetitive sequence of biochemical events, associated with spinal hyperalgesia and renal diuresis occur, then a gradual depletion of sodium, fluid and urinary metabolite would be associated with a gradual and progressive onset as displayed hypothetically in Figure 5.4.

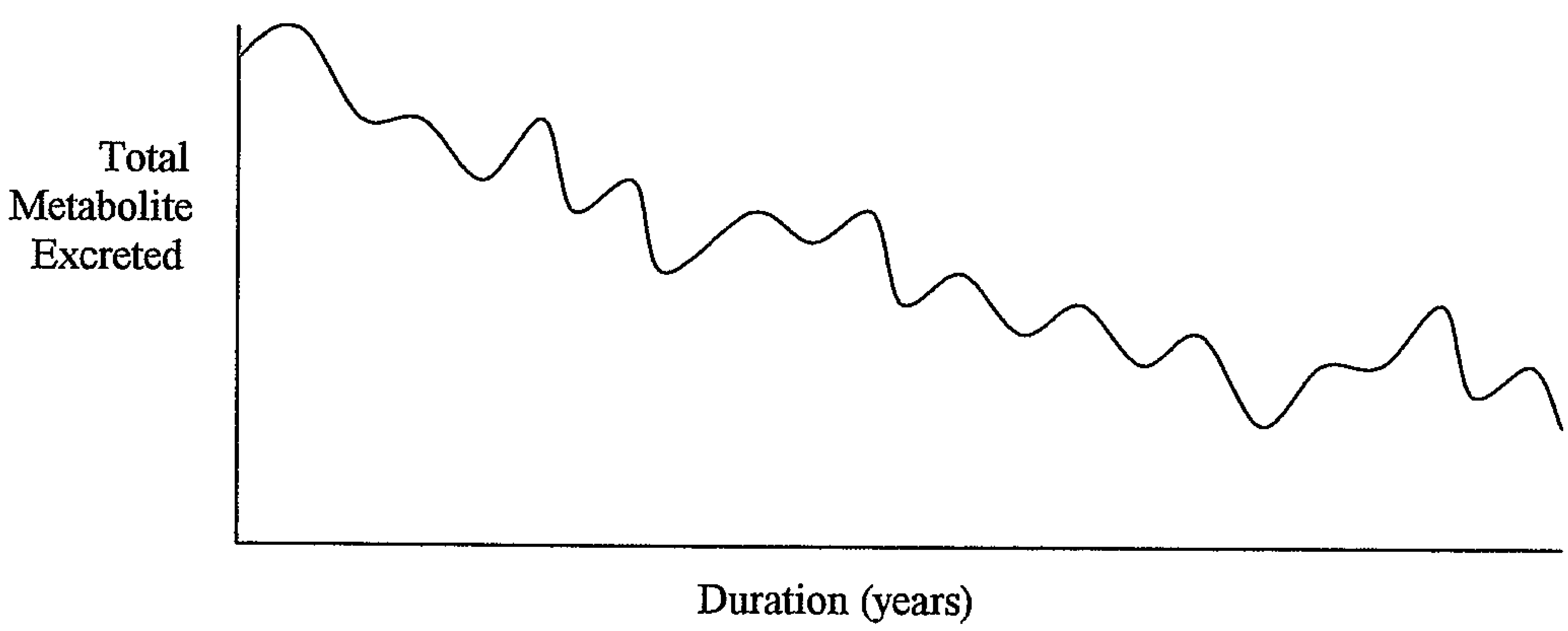

Figure 5.4. Aminoaciduria, Metabolite Depletion and Pain –proposed scheme for development of a gradual onset of chronic RDC/TMD type 1a pain. Repetitive bouts of amino acid release from body stores (muscle, liver) result from an increase in catabolic processes. The excess circulating amino acids are lost from the kidney through a nitric oxide mediated event. This prolonged repetitive process results in depletion of amino acid stores and the development of more widespread pain and increased dysregulation of multi-organ homeostasis.

These data are consistent with the aminoaciduria that occurs with trauma associated pain (Jeevanandam, 1995), together with the redistribution of the muscle and liver amino acids and HPA axis changes may contribute to the reduction in total protein and RNA in painful muscles (Young et al, 1970; Preedy et al, 1987; Pacy et al, 1988). These data therefore provide part of the biochemical basis for a gradual pain onset, and the progression of symptom severity and pain distribution, where the progressive loss of metabolites is associated with a gradual increase in pain distribution and severity.

Other factors are also associated with the reduction in muscle protein levels (Young et al, 1970; Preedy et al, 1987; Pacy et al, 1988) such as the redistribution of amino acids from muscle to liver. Illness duration is associated with reduction in total concentrations of alanine and glutamate/glutamic acid, suggesting that there may be dysregulation of the normal glutamine/alanine responses to sepsis. Glutamine is synthesised in muscle and during a septic event is released into the circulation and is taken up by the liver. The movement of glutamine from muscle to the liver is controlled by the combined increase in corticosterone with either glucagon or noradrenaline (Hasselgren 1995). Lacey & Wilmore (1990) suggested that chronic diseases allow a situation to exist where sufferers may have a "conditional deficiency" in glutamine. The fall in glutamine and its conditional deficiency may also be responsible for the increase in gastrointestinal symptoms, described in Chapter 2, as glutamine is a major energy source for enterocytes (Souba, 1991). Chronic pain may therefore result in a similar conditional deficiency state, particularly in muscle where the amino acids are redistributed to the liver. Glutamine release from muscle is also associated with changes in alanine. During cytokine mediated events alanine is released from muscle for uptake into the liver where it is used to initiate gluconeogenesis (Hasselgren 1995). This reaction is controlled by the enzyme alanine aminotransferase (ALT) and normally results in a hyperglycaemia (Hasselgren 1995). Figure 5.5 shows the basic ALT reaction and how it relates to the normal interchange of glucose and alanine between liver and muscle. If alanine, glutamic acid and α-ketoglutarate were substantially depleted due to chronic pain, this is likely to be associated with significant alterations in symptom expression as described in Chapter 2. These events may also have a negative impact on the liver's capacity to undergo gluconeogenesis. The depletion of these amino acids could therefore explain in part, why some patients have episodes of hypoglycaemia (Oles, 1978).

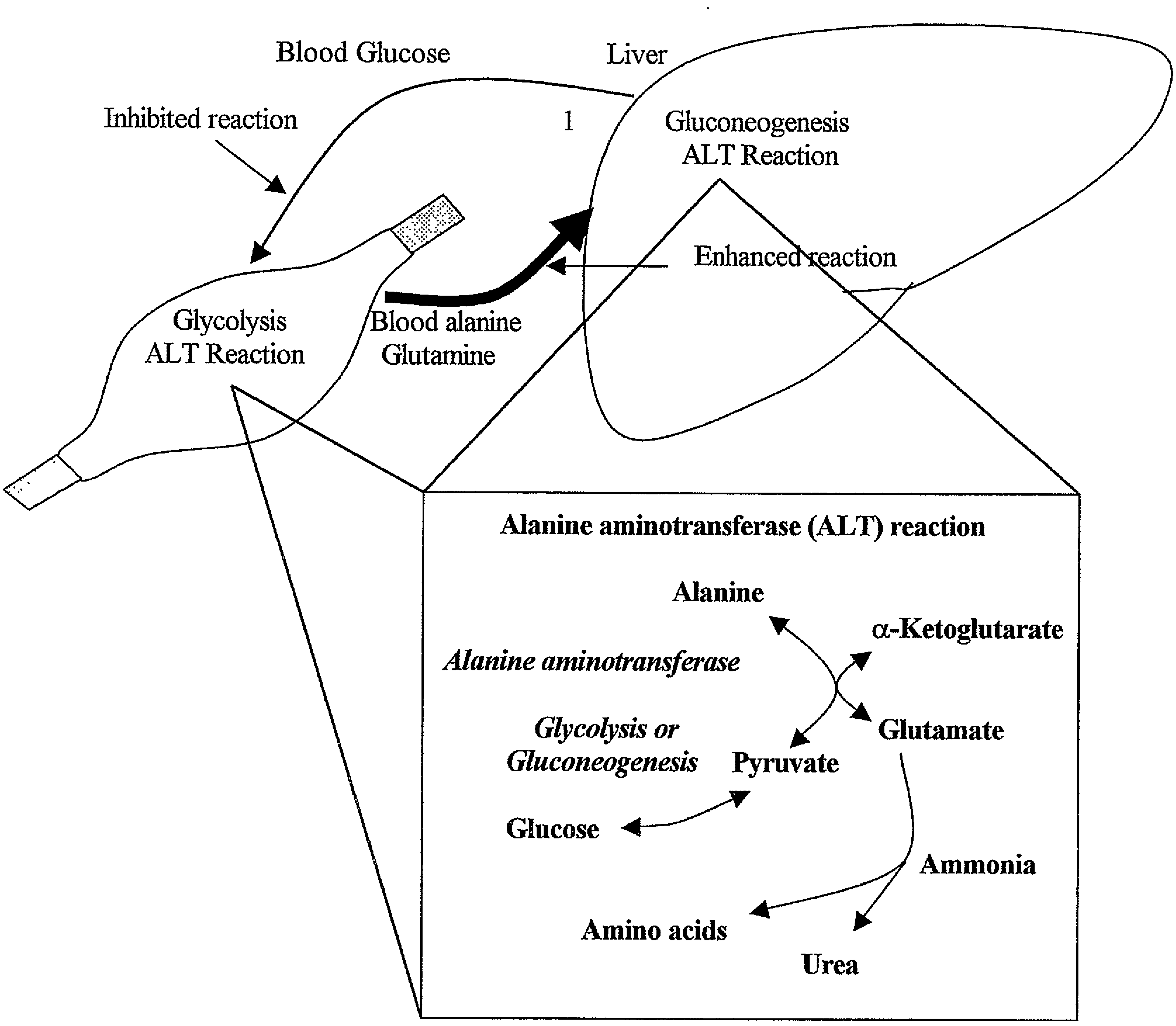

Figure 5.5. Overview of Alteration in the Glucose/Alanine and Alanine Aminotransferase (ALT) Metabolism with prolonged cytokine mediated changes.

1. In the liver alanine is used in the gluconeogenesis reaction to produce glucose which is then either stored as glycogen or enters the blood stream to maintain blood glucose levels. When a chronic cytokine response is initiated the prolonged corticosterone response results in increased movement of alanine and glutamine from muscle to the liver.
2. As muscle protein is degraded, amino acids are released, which may be metabolised and will release ammonia that is converted to glutamate and free alanine by the ALT reaction. Alanine and glutamine are released into the circulation to return to the liver to release ammonia by the ALT reaction with the ammonia being used to form amino acids or urea. In chronic cytokine responses the amino acid transporters on the muscle membrane are inhibited and the muscle develops a conditional deficiency due to the inhibition of uptake of the essential amino acids required to redevelop the normal intracellular proteins. Thus chronic pain is associated with reduced intracellular protein and RNA and illness duration was associated with increases in 3-methylhistidine. This indicates an increase in fibrillar protein degradation (actin/myosin). Once muscle glutamine is reduced, the ability of muscle to provide alanine for liver glucose production is also inhibited and may explain the increased frequency of hypoglycaemia in chronic pain patients (Oles, 1978).

The excretion rate of succinic acid was also reduced with illness duration. Injection of cytokines such as interferon (IFN) are associated with an increase in the urea cycle glucocorticoid regulated enzyme arginino-succinate synthetase, which facilitates nitric oxide production (Hattori et al, 1994), but are also associated with inhibition of complex 1 (NADH: ubiquinone oxidoreductase) of the mitochondrial respiratory chain (Geng et al, 1992). Interestingly prolonged exposure of IFN-γ and bacterial lipopolysaccharide (LPS) also inhibit mitochondrial cytochrome c oxidase and succinate-cytochrome-c reductase activity (Dijkmans & Billiau, 1991; Bolanos et al, 1994), which are likely to reduce the succinic acid levels. The progressive reduction in the excretion of succinic acid with illness duration, suggests that a cytokine and bacterial toxin interaction may be involved in the fall in urinary succinic acid concentrations. Thus, the association between membrane damaging coagulase negative staphylococci and RDC\TMD type 1a pain is supported by this observation. Baquet et al (1993) showed that succinic acid was also involved in the regulation of the enzymes associated with gluconeogenesis. With CFS patients, the reduction in urinary succinate was associated with a reduction in serum glucose (correlation between urinary succinate and serum glucose: $r=0.24$, $P<0.05$), alanine ($r=0.51$, $P<0.001$) and glutamic acid ($r=0.28$, $P<0.03$). Thus the reduction in urinary succinate is associated with dysregulation the ALT reaction and therefore glucose metabolism and the redistribution of alanine and glutamine between liver and muscle. The cytokine-driven increase in nitric oxide production facilitates inhibition of oxidative phosphorylation, reduction in muscle amino acids, the spinal hyperalgesia response, and an alteration in kidney function resulting in loss of sodium and amino acids.

The increase in release of phenylalanine with pain severity and the reduction in serum phenylalanine and tyrosine in the CFS patient cohort, shows that whilst phenylalanine release is part of the normal acute cytokine response, illness duration is associated with a fall in these amino acids. In the CFS patients, increases in serum protein levels were associated with both phenylalanine ($r=+0.27$, $P<0.008$) and tyrosine levels ($r=+0.33$, $P<0.001$). Thus the availability of catecholamine precursors falls with illness duration and would be associated with a reduction in the ability to inhibit the chronic spinal hyperalgesic, kidney diuresis and HPA axis control and is consistent with the observations of other investigators (Askanazi et al, 1980; Jeevanandam et al, 1990).

The fall in the ability of muscle to provide amino acids was also associated with a fall in hydroxyproline, also indicating a reduction in the ability of connective tissue to provide amino acids for energy. This is further supported by the observation of increased relative abundance of 3-methylhistidine an indicator of an increase in the degradation of contractile or fibrillar proteins (3-methylhistidine is a unique component of the contractile protein, actin) (Thompson et al, 1996). Therefore, illness duration is associated with evidence of reduced connective tissue and increased muscle fibre proteolysis. The reductions in the amino and organic acids from these sources strongly suggests that amino acid depletion is occurring with increasing illness duration.

5.3.2. Sex differences.

There is a 3-fold increase in the number of females in RDC\TMD type 1a pain patients who seek treatment for their pain (Schwartz, 1955; Campbell, 1958; Pedersen & Hansen, 1987). Pullinger et al (1988) found that the more severe the symptoms the higher the female: male ratio. Of those subjects with palpable muscle tenderness, 68% were female and of those with multiple site tenderness, 87% were female (Pullinger et al, 1988). Wanman & Agerberg (1986a; 1986b; 1986c; 1986d; 1986e) noted that there was an equal sex distribution until approximately 18 years of age after which female incidence increased greater than males. Agerberg and Osterberg (1974) similarly reported a 1:1 ratio in individuals of 70 years of age. These studies show an increased female to male incidence from 18 to 70 years of age, typified by an increase in number of sites and severity of pain symptoms.

In this study, jaw muscle pain was associated with a reduction in sodium levels in females. Unlike males, females are very susceptible to reduced serum sodium and hyponatremia as a result of an alteration in Na^+K^+ATPase (Arieff et al, 1976; Arieff & Fraser 1987; Ayus et al, 1988; Fraser et al, 1989). Hyponatremia and altered Na^+K^+ATPase activity are associated with cell membrane alteration in excitable cells and may result in cerebral oedema, neuronal excitotoxicity and muscle contractures. The two female sex hormones, oestrogen and progesterone, alter Na^+K^+ATPase activity in many tissues and vary during the female reproductive cycle (Davis et al, 1978; Temma et al, 1983; Labella et al, 1984; Lijnen et al, 1985; Del Castillo et al, 1987; Schwarz et al, 1988). The alteration in Na^+K^+ATPase activity is a nitric oxide mediated event (Geary et al, 1998; Hayashi et al, 1998) and that this response was exacerbated by bacterial toxins (Ikejima et al, 1998). Thus variation in female sex hormones are likely to be involved in the variation in the pain mechanisms, possibly by changes in nitric oxide and Na^+K^+ATPase activity. This increased susceptibility to alterations in sodium and nitric oxide mediated chemistry may therefore contribute to the increased severity of TMD symptoms in females.

5.3.3. Treatment approaches.

Based upon evidence from the literature and from this study, treatment approaches need to be directed at:

1. removing the aetiological agents (the toxin producing staphylococcus) and in the cases where patients have multiple influences, such as CFS and fibromyalgia, the additional agents need to be addressed.
2. Correction of amino acid loss and redistribution.
3. Correction of salt loss.
4. Correction of immune cell products.
5. Correction of catecholamine levels.
6. Assessment/ treatment or correction of genetic or other influences that may interfere with or exacerbate the response, such as organochlorine exposure and disaccharidase deficiencies.

These treatment options need to be based upon a demonstrable change and new tests need to be developed that accurately and reproducibly show these changes.

Removal of the aetiological agent should be the primary aim of the treatment of any pathogen-related disease. In TMD patients, coagulase negative staphylococci have been strongly implicated with the presence of RDC\TMD type 1a pain. In a small clinical pilot study of 20 RDC\TMD type 1a pain patients, after isolation of coagulase negative staphylococci and determination of antibiotic susceptibility, patients were prescribed an appropriate antibiotic. Nine patients (45%) were completely symptom-free after antibiotic treatment, which was associated with complete removal of toxic staphylococci (McGregor et al, 1993b). Of the remaining 11 subjects, 7 (35%) lost all pain symptoms, but these returned after completing antibiotic treatment, and this was associated with return of toxicogenic coagulase-negative staphylococci. The remaining four (20%) patients did not show any difference in symptom presentation suggesting that the cause of their pain had a different origin. A second clinical trial has been organised with independent clinicians to assess the use of nasal Bactroban[♰] application. Preliminary data suggest a similar loss of jaw muscle pain. The Staphypen toxoid vaccination data (previously mentioned) is likely to provide a superior outcome. The development of vaccines or similar anti-toxoid agents is therefore required.

However, clinical data shows that toxicogenic coagulase negative staphylococci are not the sole pathogen associated with myofascial pain. Gastrointestinal infestation by *Giardia*

[♰] Smith Kline Beecham International - Pharmaceuticals *lambda*, a parasitic protozoan, can also lead to jaw muscle pain. Giardia infestations have also been associated with a similar immune cell Th1/Th2 shift noted with toxicogenic coagulase-negative staphylococci (Djamiatun & Faubert, 1998), and increased reporting of allergies and food intolerance (Di Prisco et al, 1998). These preliminary observations warrant further investigation.

Infections with *Mycoplasma fermentans*, an L-form bacterial species commonly found in dental plaque, are also correlated with the development of TMD clicking and locking in adults (Watanabe et al, 1998). In unpublished data from the third study, the prevalence of carriage of *Mycoplasma fermentans* was associated with TMJ clicking and locking (n=64; 15 reported TMJ clicking locking; Prevalence of *Mycoplasma fermentans* in TMJ clicking/locking 46.7%; No TMJ 14.3%, $P<0.007$; Odds Ratio=5.3, $\chi^2=6.32$, $P<0.02$). *Mycoplasma fermentans* was not associated with any other major symptom. Thus, a positive association between *Mycoplasma fermentans* and TMJ dysfunction was noted in both studies and strongly suggests that these relationships be further investigated. These data suggest that a number of different pathogens are associated with the development of TMD symptoms, with particular pathogens being associated with different symptom constellations.

Amino acid supplementation, to overcome the whole body and tissue amino acid redistribution and the reduction in catecholamine precursors as a result of the disease process, needs to be investigated. Preliminary pilot study data suggests that amino acid supplementation, without addressing the issue of pathogen control, is of considerable benefit with the neck/shoulder pain groups, but unless used in conjunction with salt replacement is less effective with jaw muscle pain. The correction of the hyponatremic-like symptoms appears to be of prime importance in the female RDC/TMD type 1a pain patients. There are treatment protocols currently available for salt replacement but these may need to be modified in the light of the information found in this study, as they do not address the issue of reduced catecholamine precursors or the possible enhanced nitric oxide response. It is worthy of note that TMD patients have lower blood pressures than controls (de Abreu et al, 1993), that drug-associated dysregulation of dopamine metabolism is associated with TMD symptoms (Lynch et al, 1961; Pertoutka et al, 1988) and the use of noradrenergic reuptake inhibiting (antidepressant) medication reduces pain expression in TMD patients (Tura & Tura, 1990). Modulation of the various immune products is also important when considering these treatment options. An examination of the role of histamine in development of

RDC/TMD type 1a pain is required. Controlled clinical trials need to be established to examine treatment effectiveness, to determine the appropriate treatment protocols.

The accumulation of the organochlorine pesticide residues in the RDC/TMD type 1a pain patients has a profound central nervous influence upon both neurotransmission and glucose availability. In this study, increasing Dieldrin levels were strongly associated with increases in TMD symptoms in CFS males. Dieldrin accumulation was associated with evidence of a nitric oxide mediated event, increased lipid turnover (ethanolamine) and the accumulation of the non-host s-methylcysteine. DDE was also positively associated with jaw muscle pain as well as increases in serum glucose, s-methylcysteine, ethanolamine, ALT, basophils and the urinary tyrosine: leucine ratio. These agents appear to accumulate with the disease as well as with age in the general population. Therapeutic measures to either remove the organochlorine pesticide residues or to combat the effects of these compounds are required. The inhibition of GABA receptor activity induced by the organochlorines may be reduced with the use of drugs such as gabapentin, benzodiazapams and valporate sodium, which increase GABA activity by various mechanisms.

An interesting observation was made by Kapral (1976), where alteration in the levels of various fatty acids was associated with alteration in toxicity of staphylococcal δ-toxin. Fatty acids greater than 20 carbons, inhibited δ-toxin induced haemolysis, whilst fatty acids between C14 and C18, increased the rate of haemolysis. The fatty acids, C15:0, C16:0 and C17:0 gave the highest level of haemolysis. This appears to result from the ion channel actions of δ-toxin in bacterial cells which have cell membranes dominated by C17:0 fatty acids. If the cell walls are thicker, then δ-toxin is not able to penetrate the membrane to act as an ion channel. In the laboratory the consumption of very long chain unsaturated fatty acids (evening primrose oil C20 and fish oil C22-C26) resulted in inhibition of haemolysis in the standard haemolysis assays. Fish oil supplementation has the additional benefit of reducing neutrophil superoxide production (Luostarinen et al, 1996) and may reduce the long-term induction of tissue damage noted in the RDC/TMD type 1a patients. A study is needed in RDC/TMD type 1a patients to assess the effects of fish oil supplementation on both coagulase negative staphylococcal toxicity and immune cell changes.

5.4. Summary.

This chapter summarises the research findings and proposes that toxicogenic coagulase negative staphylococcus species are one major cause of myofascial pain and represent a disease entity. Dysregulation of excitatory amino acids, adrenergic precursors, proteolysis/protein synthesis, fluid and sodium balance and inflammatory cell distribution and evidence of tissue damage were noted and the mechanisms leading the development of TMD have been proposed. New assessment procedures are required for accurate clinical evaluation associated with treatment methods to address these abnormal bacterial and biochemical mechanisms.

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APPENDIX I

PAIN QUESTIONNAIRE.

This questionnaire is designed to give very comprehensive details, which will help in the diagnosis of your problem. It is very important that you do not leave out any details, no matter how irrelevant they may seem or whether you feel they may be caused by other factors. They may be just the facts that solve the problem.

SURNAME:(Mr,Mrs,Ms,Miss,Dr)	
Other Names	Date of Birth
Home Address	Postcode
Telephone: Home	Work
Marital Status: [ ]Single, [ ]Married, [ ]Seperated, [ ]Divorced, [ ]Defacto, [ ]Widowed.	
Occupation	
Partners Occupation	
Name and Address of referring Practitioner	
Name and Address of your Medical Practitioner	
What is your major pain complaint?	
Is the pain problem subject to a compensation claim? Yes[ ]	No[ ]

Where was the very first place you ever had pain? Face Neck Shoulder Low Back Other	
If "Other" where did it first occur?	
How long ago did the very first symptoms appear?	
Was the onset of the first pain sudden or gradual	
If your first pain symptoms were not in your face how long after the first pain did the facial pain start?	
If not the first pain, was onset of facial pain sudden or gradual	
Place a cross beside anything that occurred around the time of first onset of the pain: I was taking Antibiotics Antidepressants Pills Drugs Vitamins or tonics Other Received trauma to the face, neck or back (car accident etc) I had prolonged dental or orthodontic treatment Fever or infection (Respiratory, Urinary, Skin, Genital, Viral, etc) I had another illness I had a general anaesthetic I was in a stressful situation I had radiotherapy, Other not mentioned	
More Detail if required	
Within 12 months prior to onset of your first pain symptoms did you start a new sexual relationship?	
Does your sexual partner have any muscular aches and pains or similar problems ? Yes No	
If yes, where are their pain problems ? Face Neck Shoulder Lower Back Other	
More detail if required	
Did your condition start in the summer/autumn winter/spring.	
Does your job involve the use of the painful muscles? Yes No	
If "yes", which muscles and what is the job activity?	
What do you think was the cause of your pain?	

To assess the pain severity of the worst area. Place the most appropriate number in the box. (0=Nil, 1=Mild, 2=Moderate, 3=Severe) Which area are you rating? .... 1. How intense is your usual level of pain? 2. How often does the pain generally occur? irregular 1/mnth 1/week 1/day 1/hour constant 3. How long does the pain generally last? irregular 1/minute 1/hour 1/day 1/week constant	
Is your pain worse : When using the muscles (Chewing etc)? When stretching the muscles (Opening mouth wide)? When pressing on the muscles? When under stress? When the weather is cold or you get cold? During summer/autumn? During winter/spring?	
Was there a change in your general health when the pain started? If "yes", what was the change?	
Are you receiving treatment for a medical condition?	
If "yes", what is the medical condition and what is the treatment?	
Please list any other operation, accidents, serious illnesses or medical investigations you have had: Event. Approx. Date.	
Please list below the names of all antibiotics, antidepressants, pills, drugs, medicines, tablets or vitamins you are taking at present?	
Have you had a reaction to any drugs? if "yes", what drug/s and what was the reaction/s?	

Have you had a general anaesthetic? If yes, did you have any complications and what were they?	
Do you visit your medical practitioner often? If yes, why?	
Were you often sick as a child? If yes, why?	
Do you regularly handle domestic pets or animals or livestock?	
Have you ever had food poisoning?	
What medical, dental, chiropractic, osteopathic, naturopathic or other treatment have you already had?	
Did any of these treatments help? Yes If "Yes", which ones helped? No Don't Know	
Is there anything else you can tell us that would help us to understand your problem ?	

Place a cross beside any of the symptoms which frequently occur to you.	
Headaches If yes are they Migraines Tension type Cluster type Sinus related	
Weakness or paralysis of muscles If yes do they occur in the Face Neck or shoulder Back Limb	
Uncontrolled muscle movements or twitches If yes do they occur in the Face Neck or shoulder Back Limb	
Easily fatigueable muscles If yes do they occur in Face Neck or shoulder Back Limb	
Frequent muscle cramps If yes are they Writers cramp Carpopedal spasm Limb	
Grinding or clenching your teeth	
Painful/stiff joints in the morning	
Arthritis	
Joint nodules or spurs	
Locking or clicking jaw joint	
Sinusitis or stuffiness in the nose	
Staphylococcal nose or skin infections	
Sore throats/nasal infections	
Earaches	
Tinnitis or Noises in the ear	
Dizziness or Fainting spells	
Problems with sleeping	
Pains in heart or chest	
Heart pounding, racing or palpitations	
Trouble getting your breath	
Exertional chest pain/breathlessness	
Gastric reflux	
Nausea or upset stomach	
Intermittant unexplained diarrhoea	
Irritable bowel syndrome	
Constipation	
Recurrent oral ulceration	
Abdominal fullness or swelling	
Abdominal pain or tenderness	
Suprapubic or subnavel tenderness/pain	
Frequent urination	
Burning or uncomfortable urination	
Urgent urination	
Low back pain	
Menstrual pain	
Other menstrual/genital tract problems	

Recurrent feverishness	
Sore lymph glands	
Loss of feeling/numbness of the skin	
Hypersensitivity of the skin	
Hot spells or hot hands	
Cold spells or cold hands	
Dermatitis or skin rashes	
Skin infections	
Skin peeling on the hands or feet	
Allergies	
Excessive or unusual hair loss	
Details or further explanations if necessary	
What are your parents racial or ethnic backgrounds? (ie, Greek, Anglosaxon, Celtic, European Jew, Negro, Australian Aboriginal, etc.) Father? Mother?	

Do you have or is there a family history of any of the following diseases or problems; If in the family number the column marked with an "F" and put details at the bottom of the page; if you have the problem tick the column marked with the "U". If you have not heard of the disease ask about it. Family constitutes your blood relatives and those of your partner.

Problem	U.	F.	Problem	U.	F.	Problem	U.	F.
Acromegaly			Thyroid problems			Parathyroid gland problems		
Adrenal Gland problems			Diabetes Mellitus			Hypoglycemia		
Staphylococcal Infections			Toxic Shock Sydrome			Impetigo		
Scarlet Fever			Scalded skin syndrome			Ovarian cysts		
Menstrual problems			Premenstrual tension			Eclampsia/Pre-eclampsia		
Endometriosis			Hysterectomy			Cervicitis		
Vulvovaginitis			Urinary Tract infections			Urethritis		
Urethral syndrome			Cystitis			Pyelitis		
Pyelonephritis			Kidney stones			Glomerulonephritis		
Bladder problems			Kidney problems			Renal tubular acidosis		
Polycystic Kidney			Reiter's syndrome			Renal or Kidney failure		
High blood pressure			Low Blood Pressure			Peptic or Duodenal Ulcer		
Irritable colon			Disaccharidase deficiency			Milk allergy		
Gout			Pseudogout			Rheumatoid arthritis		
Ankylosing Spondylitis			Psoriasis			Lupus erythematosis		
Sarcoidosis			Osteoarthritis			Slipped or herniated Disc		
Scoliosis			Sciatica			Fibrositis or Fibromyalgia		
Osteoporosis			Anaemia			Asthma		
Chronic sinusitis			Recurrent throat infection			Glycogen storage disease		
Sicca syndrome			Sjogren's syndrome			Epilepsy		
Cancer			Phaeochromocytoma			Other not mentioned		

Family details and further information if necessary?

1
2
3
4
5
6
7
8

CLINICAL EXAMINATION FORM.

Name:	Date:	
Muscle Palpation. 1=Pos. 0=Neg.	Right	Left
Glabella	Control	
Orbicularis Occuli		
Levator Labii Superioris		
Anterior Temporalis		
Deep Temporalis		
Middle Temporalis		
Posterior Temporalis		
Deep Masseter		
Anterior Masseter		
Body Masseter		
Inferior Masseter		
Anterior Digastric		
Posterior Digastric		
Medial Pterygoid		
Occipitalis		
Frontalis		
Vertex	Control	
Temporalis tendon (Intraoral)		
Medial pterygoid (Intraoral)		
Mastoid process (Control)		
Sternocleidomastoid		
Anterior Trapezius		
Mid-Shoulder Trapezius		
Back Trapezius		
Functional Assessment		
Reciprocal Click		
Reproducible click		
Non-reproducible click		
Crepitus		
Maximum opening(interincisor)		mm
Passive stretch		mm
Restriction or rigidity on opening		
Jerky opening or closing		
Deviation on opening (>2mm)		
Lateral deviation at full opening		
Limited protrusion		
Limited lateroprotrusion		
Clinically can lock open (subluxate)		
Clinically does condyle not translocate		
Tooth wear 0, 1=Min., 2=Mod., 3=Gross		
Notes		

American Academy of Rheumatology Fibromyalgia Tenderpoints.

APPENDIX II

ROYAL NORTH SHORE HOSPITAL

CONSENT FORM TO PARTICIPATE IN A RESEARCH PROJECT

I..
of...postcode.................
have been invited to participate in a research project titled:

Evaluation Of Metabolic Changes In Myalgic Encephalomyelitis/Chronic Fatigue Syndrome (ME/CFS).

In relation to this project I have been informed of the following points;

1. Approval has been given by the Medical Research Ethics Committee of Royal North Shore Hospital.

2. The aim of the study is to determine whether changes in metabolism occur in ME/CFS suffers. We are investigating whether this change can be measured by changes in blood and urinary substances which have been recently identified in a study by Newcastle University. We want to find out if this reported change does occur in a larger population and whether these changes are unique to people with ME/CFS or can also occur in people : 1) with an illness with similar symptoms to ME/CFS but with a definite test to identify the cause eg Ross River Virus, and 2) people who have depression. We also need to compare the results to : 3) people who are basically healthy and have not been diagnosed with ME/CFS or another illness. This group will be our control group.

3. The results of the study may or may not be of direct benefit to my medical management.

4. The procedure will involve, in the majority of cases, a one off collection of urine and blood samples, and completion of three questionnaires. You may be asked to be a part of a small number of participants in the study to take part in a 10 week follow-up. The follow up consists of a) every two weeks (5 times in all) a urine sample will be taken, and b) two short questionnaires and a special test on a computer to measure memory and concentration.

5. I can refuse to take part in this project or withdraw from it at any time without it affecting my medical care.

6. The results of any tests or information regarding my medical history will not be published so as to reveal my identity.

7. Once the results of the study are published I can obtain a copy of the published papers and/or ask for the papers to be forwarded to my consulting doctor. I understand that the results may not necessarily agree with my current beliefs about ME/CFS.

8. If I have any concerns about the project or my involvement I understand that I can contact Ms Leigh Hoskin Department of Health Promotion and Education on 438 7763.

After considering all these points I accept the invitation to participate in this project.
I also state that I have/have not participated in any other research project in the past 3 months.
If I have the details are as follows...

SIGNATURE...WITNESS.......................................
(Of patient/volunteer) (Please print name)

DATE..SIGNATURE...
(witness)

APPENDIX III

Background Questionnaire

ROYAL NORTH SHORE HOSPITAL AND THE UNIVERSITY OF NEWCASTLE RESEARCH STUDY

BACKGROUND QUESTIONNAIRE.

This questionnaire will ask you background information questions. Please answer these questions honestly and accurately. The information given is confidential. Please tick the box or blacken the circle where appropriate, or write your comments in the spaces provided. Please take your time to complete this questionnaire.

1. What is the Postcode of your home address:

   ____

2. What is the Postcode of your School / Work address:

   ____

3. Are you

   ☐ Male
   ☐ Female

4. What is your Date of Birth:

   / /19

5. What is your age now

   _______ years _______ months

6. Are you presently studying ?

- ☐ Yes
- ☐ **No, go to Question 7**

If yes, do you study:

- ☐ Part time at school
- ☐ Part time by correspondence
- ☐ Part time at TAFE
- ☐ Part time - Pathways
- ☐ Part time at University
- ☐ Full time at school
- ☐ Full time at University
- ☐ Full time at TAFE
- ☐ Full time by correspondence
- ☐ Other ______________

7. Are you presently working ?

- ☐ Yes
- ☐ **No, go to Question 8**

If yes, do you work

- ☐ Part time (paid)
- ☐ Part time (voluntary)
- ☐ Full time (paid)
- ☐ Full time (voluntary)
- ☐ Casual (paid)
- ☐ Casual (voluntary)

8. What are your means of financial support ?

- ☐ Disability Allowance
- ☐ Sickness benefits
- ☐ Unemployment benefits
- ☐ Supported by family
- ☐ Supported by partner
- ☐ Supported by self
- ☐ Other ______________

9. Are you a member of a Support Group?

☐ Yes
☐ **No, go to Question 11**

If Yes, which support group?

____

10. When did you first join the Support Group ?

_______ Year _______ Season

11. Have you been diagnosed as having:

Chronic Fatigue Syndrome (CFS)/ Myalgic Encephalomyelitis (ME)?	☐ Yes	No ☐
Ross River Virus (R.R.V.)?	☐ Yes	No ☐
Clinical Depression?	☐ Yes	No ☐

If No to the above, go to Question 28

12. When did you first begin to feel unwell ?

_______ year _______ season

13. When were you first diagnosed ?

_______ year

14. Who made the diagnosis ?

☐ Psychologist / Psychiatrist
☐ Medical Doctor / GP
☐ Medical Doctor / Specialist / Professor
☐ Alternative Practitioner
☐ Other, please specify _________________________

15. Was the onset of your illness:

☐ Sudden
☐ Gradual

16. Please indicate which of the following medications / treatments / activities you have tried and how effective they were? (please tick the appropriate box)

		Made things worse	No Change	Works for a while	Made things better
☐	Gamma Globulin	☐	☐	☐	☐
☐	Transfer factor	☐	☐	☐	☐
☐	Elimination diet	☐	☐	☐	☐
☐	Anti depressants	☐	☐	☐	☐
☐	Prozac	☐	☐	☐	☐
☐	Prothiadin	☐	☐	☐	☐
☐	Vibra tabs	☐	☐	☐	☐
☐	Antifungals	☐	☐	☐	☐
☐	Vitamin C	☐	☐	☐	☐
☐	Vitamin B12	☐	☐	☐	☐
☐	Naprosyn	☐	☐	☐	☐
☐	Total Rest	☐	☐	☐	☐
☐	Cortisone	☐	☐	☐	☐
☐	Counselling	☐	☐	☐	☐
☐	Magnesium	☐	☐	☐	☐
☐	Enemas	☐	☐	☐	☐
☐	Pain killers	☐	☐	☐	☐
☐	Sleeping tablets	☐	☐	☐	☐
☐	Physiotherapy	☐	☐	☐	☐
☐	Resting when necessary	☐	☐	☐	☐
☐	Antibiotics	☐	☐	☐	☐
☐	Rehabilitation programme	☐	☐	☐	☐
☐	Behaviour therapy	☐	☐	☐	☐
☐	Other,	☐	☐	☐	☐

Please specify ____

____

____

____

17. What do you think triggered your illness?

____
____
____
____
____
____

18. Please indicate which of the following practitioners you have visited for your ME/CFS, Clinical depression or RRV and how helpful they were:

		Made things worse	No Change	Works for a while	Made things better
☐	Chiropractor	☐	☐	☐	☐
☐	Naturopath	☐	☐	☐	☐
☐	Osteopath	☐	☐	☐	☐
☐	Social Worker	☐	☐	☐	☐
☐	Psychologist	☐	☐	☐	☐
☐	Masseuse	☐	☐	☐	☐
☐	Acupuncturist	☐	☐	☐	☐
☐	Aromatherapist	☐	☐	☐	☐
☐	Herbalist	☐	☐	☐	☐
☐	Iridologist	☐	☐	☐	☐
☐	Homeopath	☐	☐	☐	☐
☐	Psychiatrist	☐	☐	☐	☐
☐	Hypnotherapist	☐	☐	☐	☐
☐	Gynaecologist	☐	☐	☐	☐
☐	Paediatrician	☐	☐	☐	☐
☐	Neurologist	☐	☐	☐	☐
☐	Clinical Ecologist	☐	☐	☐	☐
☐	Physiotherapist	☐	☐	☐	☐
☐	Dietitian	☐	☐	☐	☐
☐	Chinese Medicine Doctor	☐	☐	☐	☐
☐	Podiatrist	☐	☐	☐	☐
☐	Occupational Therapist	☐	☐	☐	☐

	Made things worse	No Change	Works for a while	Made things better
☐ Medical Practitioner	☐	☐	☐	☐
Gastroenterologist	☐	☐	☐	☐
Lymphatic Drainage practitioner	☐	☐	☐	☐
Other practitioner	☐	☐	☐	☐

Please specify ______________________________

19. Please indicate which of the following tests you have had:

- ☐ EEG (Electro-Encephalogram / Brain Scan)
- ☐ ECG (Electro-Cardiogram / Heart)
- ☐ Malaria
- ☐ Dangue Fever
- ☐ Glandular Fever
- ☐ HIV
- ☐ Stool Test
- ☐ TFT (Thyroid Function Test)
- ☐ Cell Lymphocytes Subtests
- ☐ Antibodies Blood Test
- ☐ Rheumatoid Arthritis
- ☐ IGG / IGM (Test for Ross River Virus)
- ☐ Lyme Disease
- ☐ Thyroid tests
- ☐ Allergy tests
- ☐ Hair analysis
- ☐ Blood tests for pesticide
- ☐ Vitamin level tests
- ☐ Neurological tests
- ☐ T Cell tests
- ☐ Liver function test
- ☐ Full blood count
- ☐ Other, please specify

____

20. Below are some statements with which some people agree and other disagree. Please read each statement and ***blacken the circle*** for the response most appropriate for you. ***Blacken only one circle for each statement*** and do not skip any. There is no right or wrong answer.

STATEMENTS	STRONGLY DISAGREE	DISAGREE	SOMEWHAT DISAGREE	NEUTRAL	SOMEWHAT AGREE	AGREE	STRONGLY AGREE
1. There is someone I feel close to who makes me feel secure	①	②	③	④	⑤	⑥	⑦
2. I belong to a group in which I feel important	①	②	③	④	⑤	⑥	⑦
3. People let me know that I do well at my work (job, study, homemaking)	①	②	③	④	⑤	⑥	⑦
4. I can't count on my relatives and friends to help me with problems	①	②	③	④	⑤	⑥	⑦
5. I have enough contact with the person who makes me feel special	①	②	③	④	⑤	⑥	⑦
6. I spend time with others who have the same interests that I do	①	②	③	④	⑤	⑥	⑦
7. There is little opportunity in my life to be giving and caring to another person	①	②	③	④	⑤	⑥	⑦
8. Others let me know that they enjoy working with me (jobs, committees, projects)	①	②	③	④	⑤	⑥	⑦
9. There are people available if I needed help over an extended period of time	①	②	③	④	⑤	⑥	⑦
10. There is no one to talk to about how I am feeling	①	②	③	④	⑤	⑥	⑦
11. Among my group of friends we do favours for each other	①	②	③	④	⑤	⑥	⑦
12. I have the opportunity to encourage others to develop their interests and skills	①	②	③	④	⑤	⑥	⑦
13. My family lets me know that I am important	①	②	③	④	⑤	⑥	⑦
14. I have relatives or friends that will help me out even if I can't pay them back	①	②	③	④	⑤	⑥	⑦

STATEMENTS	STRONGLY DISAGREE	DISAGREE	SOMEWHAT DISAGREE	NEUTRAL	SOMEWHAT AGREE	AGREE	STRONGLY AGREE
15. When I am upset there is someone I can be with who lets me be myself	①	②	③	④	⑤	⑥	⑦
16. I feel no one has the same problem as I	①	②	③	④	⑤	⑥	⑦
17. I enjoy doing little 'extra' things that make another person's life more pleasant	①	②	③	④	⑤	⑥	⑦
18. I know that others appreciate me as a person	①	②	③	④	⑤	⑥	⑦
19. There is someone who loves and cares about me	①	②	③	④	⑤	⑥	⑦
20. I have people to share social events and fun activities with	①	②	③	④	⑤	⑥	⑦
21. I am responsible for helping provide for another person's needs	①	②	③	④	⑤	⑥	⑦
22. If I need advice there is someone who would assist me to work out a plan for dealing with the situation	①	②	③	④	⑤	⑥	⑦
23. I have a sense of being needed by another person	①	②	③	④	⑤	⑥	⑦
24. People think that I'm not as good a friend as I should be	①	②	③	④	⑤	⑥	⑦
25. If I got sick, there is someone to give me advice about caring for myself	①	②	③	④	⑤	⑥	⑦

21. What has been the most distressing part of having C.F.S., R.R.V, Clinical Depression ?

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____
____

22. What has been the most helpful event, person or thing for your C.F.S., R.R.V, Clinical Depression ?

____
____
____

23. Who of the following do you think need more information on your particular illness (C.F.S./ R.R.V./ Clinical Depression)? (Please tick as many as apply)

- ☐ Local GP
- ☐ Medical Specialists
- ☐ Other Health Professionals
- ☐ Self
- ☐ Your family
- ☐ Your friends
- ☐ School Teachers
- ☐ School Nurse
- ☐ Department of Social Security
- ☐ Employers
- ☐ Occupational Health & Safety Officer / First Aid Officer at Work
- ☐ General public
- ☐ Other, please list

____
____

24. In which of the following areas would you like more information ?
(Please tick as many as apply)

- ☐ Basic information on C.F.S. / R.R.V. / Clinical Depression
- ☐ Treatments
- ☐ Tests
- ☐ Management skills
- ☐ Coping strategies
- ☐ Strategies to deal with pain
- ☐ Tips for school/work
- ☐ Tips for living with uncertainty
- ☐ Sexuality
- ☐ Body image
- ☐ Ways of dealing with relationship issues
- ☐ How to manage depression
- ☐ How to manage anger / frustration
- ☐ Ways of dealing with family
- ☐ Tips for work
- ☐ Dealing with Doctors and Health Professionals
- ☐ Stress
- ☐ Department of Social Security
- ☐ Latest Developments / Research
- ☐ Other ____

25. Can you comment on your experience with health professionals since diagnosis ? (Please be as frank as you wish).

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26. Can you comment on your experience with School teachers / work personnel since your diagnosis ? (Please be as frank as you wish).

27. How do you think Health Professionals can make things easier for you ?

28. Below is a list of problems people sometimes have. Please read each on carefully, ***and blacken the circle that best describes HOW MUCH THAT PROBLEM HAS DISTRESSED OR BOTHERED YOU (in general) WHEN IT WAS PRESENT OVER THE PAST 12 MONTHS.*** Also, ***blacken the circle that best describes APPROXIMATELY HOW FREQUENTLY THE PROBLEM WAS PRESENT OVER THE PAST 12 MONTHS.*** Answer both questions for each problem and ***do not*** skip any items. If you change your mind, put a cross through your first choice and blacken the circle of your new choice. Read the example before beginning, and if you have any questions please ask about them.

HOW MUCH WERE YOU DISTRESSED BY:					EXAMPLE	HOW OFTEN WAS THE PROBLEM PRESENT:				
NOT AT ALL	A LITTLE BIT	MODER-ATELY	QUITE A BIT	EXTRE-MELY	PROBLEM	ALL OF THE TIME	MOST OF THE TIME	SOME OF THE TIME	RARELY	NEVER
⓪	①	②	●	④	Bodyaches	⓪	●	②	③	④

HOW MUCH WERE YOU DISTRESSED BY:					PROBLEM	HOW OFTEN WAS THE PROBLEM PRESENT:				
NOT AT ALL	A LITTLE BIT	MODER-ATELY	QUITE A BIT	EXTRE-MELY		ALL OF THE TIME	MOST OF THE TIME	SOME OF THE TIME	RARELY	NEVER
⓪	①	②	③	④	Headaches	⓪	①	②	③	④
⓪	①	②	③	④	Nervousness or shakiness inside	⓪	①	②	③	④
⓪	①	②	③	④	Repeated unpleasant thoughts that won't leave your	⓪	①	②	③	④
⓪	①	②	③	④	Faintness or dizziness	⓪	①	②	③	④
⓪	①	②	③	④	Loss of sexual interest or pleasure	⓪	①	②	③	④
⓪	①	②	③	④	Feeling critical of others	⓪	①	②	③	④
⓪	①	②	③	④	The idea that someone else can control your thoughts	⓪	①	②	③	④
⓪	①	②	③	④	Feeling that others are to blame for most of your troubles	⓪	①	②	③	④
⓪	①	②	③	④	Trouble remembering things	⓪	①	②	③	④
⓪	①	②	③	④	Worried about sloppiness or carelessness	⓪	①	②	③	④
⓪	①	②	③	④	Feeling easily annoyed or irritated	⓪	①	②	③	④
⓪	①	②	③	④	Pains in heart or chest	⓪	①	②	③	④
⓪	①	②	③	④	Feeling afraid in open spaces or on the streets	⓪	①	②	③	④
⓪	①	②	③	④	Feeling low in energy or slowed down	⓪	①	②	③	④
⓪	①	②	③	④	Thoughts of ending your life	⓪	①	②	③	④
⓪	①	②	③	④	Hearing voices that other people do not hear	⓪	①	②	③	④

HOW MUCH WERE YOU DISTRESSED BY:					PROBLEM	HOW OFTEN WAS THE PROBLEM PRESENT:				
NOT AT ALL	A LITTLE BIT	MODER-ATELY	QUITE A BIT	EXTRE-MELY		ALL OF THE TIME	MOST OF THE TIME	SOME OF THE TIME	RARELY	NEVER
⓪	①	②	③	④	Trembling	⓪	①	②	③	④
⓪	①	②	③	④	Feeling that most people cannot be trusted	⓪	①	②	③	④
⓪	①	②	③	④	Poor appetite	⓪	①	②	③	④
⓪	①	②	③	④	Crying easily	⓪	①	②	③	④
⓪	①	②	③	④	Feeling shy or uneasy with the opposite sex	⓪	①	②	③	④
⓪	①	②	③	④	Feelings of being trapped or caught	⓪	①	②	③	④
⓪	①	②	③	④	Suddenly scared for no reason	⓪	①	②	③	④
⓪	①	②	③	④	Temper outbursts that you cannot control	⓪	①	②	③	④
⓪	①	②	③	④	Feeling afraid to go out of your house alone	⓪	①	②	③	④
⓪	①	②	③	④	Blaming yourself for things	⓪	①	②	③	④
⓪	①	②	③	④	Pains in the lower back	⓪	①	②	③	④
⓪	①	②	③	④	Feeling blocked in getting things done	⓪	①	②	③	④
⓪	①	②	③	④	Feeling lonely	⓪	①	②	③	④
⓪	①	②	③	④	Feeling blue	⓪	①	②	③	④
⓪	①	②	③	④	Worrying too much about things	⓪	①	②	③	④
⓪	①	②	③	④	Feeling no interest in things	⓪	①	②	③	④
⓪	①	②	③	④	Feeling fearful	⓪	①	②	③	④
⓪	①	②	③	④	Your feelings being easily hurt	⓪	①	②	③	④
⓪	①	②	③	④	Other people being aware of your private thoughts	⓪	①	②	③	④
⓪	①	②	③	④	Feeling others do not understand you or are unsympathetic	⓪	①	②	③	④
⓪	①	②	③	④	Feeling that people are unfriendly or dislike you	⓪	①	②	③	④
⓪	①	②	③	④	Having to do things very slowly to ensure correctness	⓪	①	②	③	④
⓪	①	②	③	④	Heart pounding or racing	⓪	①	②	③	④
⓪	①	②	③	④	Nausea or upset stomach	⓪	①	②	③	④
⓪	①	②	③	④	Feeling inferior to others	⓪	①	②	③	④
⓪	①	②	③	④	Soreness of your muscles	⓪	①	②	③	④
⓪	①	②	③	④	Feeling that you are watched or talked about by others	⓪	①	②	③	④
⓪	①	②	③	④	Trouble falling asleep	⓪	①	②	③	④
⓪	①	②	③	④	Having to check and double-check what you do	⓪	①	②	③	④

HOW MUCH WERE YOU DISTRESSED BY:					PROBLEM	HOW OFTEN WAS THE PROBLEM PRESENT:				
NOT AT ALL	A LITTLE BIT	MODER-ATELY	QUITE A BIT	EXTRE-MELY		ALL OF THE TIME	MOST OF THE TIME	SOME OF THE TIME	RARELY	NEVER
⓪	①	②	③	④	Difficulty making decisions	⓪	①	②	③	④
⓪	①	②	③	④	Feeling afraid to travel on buses, subways or trains	⓪	①	②	③	④
⓪	①	②	③	④	Trouble getting your breath	⓪	①	②	③	④
⓪	①	②	③	④	Hot or cold spells	⓪	①	②	③	④
⓪	①	②	③	④	Having to avoid certain things, places, or activities because they frighten you	⓪	①	②	③	④
⓪	①	②	③	④	Your mind going blank	⓪	①	②	③	④
⓪	①	②	③	④	Numbness or tingling in parts of your body	⓪	①	②	③	④
⓪	①	②	③	④	A lump in your throat	⓪	①	②	③	④
⓪	①	②	③	④	Felling hopelessness about the future	⓪	①	②	③	④
⓪	①	②	③	④	Trouble concentrating	⓪	①	②	③	④
⓪	①	②	③	④	Feeling weak in parts of your body	⓪	①	②	③	④
⓪	①	②	③	④	Feeling tense or keyed up	⓪	①	②	③	④
⓪	①	②	③	④	Heavy feelings in your arms or legs	⓪	①	②	③	④
⓪	①	②	③	④	Thoughts of death or dying	⓪	①	②	③	④
⓪	①	②	③	④	Overeating	⓪	①	②	③	④
⓪	①	②	③	④	Feeling uneasy when people are watching or talking about you	⓪	①	②	③	④
⓪	①	②	③	④	Having thoughts that are not your own	⓪	①	②	③	④
⓪	①	②	③	④	Having urges to beat, injure or harm someone	⓪	①	②	③	④
⓪	①	②	③	④	Awakening in the early morning	⓪	①	②	③	④
⓪	①	②	③	④	Having to repeat the same actions such as touching, counting, or washing	⓪	①	②	③	④
⓪	①	②	③	④	Sleep that is restless or disturbed	⓪	①	②	③	④
⓪	①	②	③	④	having urges to break or smash things	⓪	①	②	③	④
⓪	①	②	③	④	having ideas or beliefs that others do not share	⓪	①	②	③	④
⓪	①	②	③	④	Feeling very self-conscious with others	⓪	①	②	③	④
⓪	①	②	③	④	Feeling uneasy in crowds, such as shopping or at a movie	⓪	①	②	③	④
⓪	①	②	③	④	Feeling everything is an effort	⓪	①	②	③	④
⓪	①	②	③	④	Spells of terror or panic	⓪	①	②	③	④

HOW MUCH WERE YOU DISTRESSED BY:					PROBLEM	HOW OFTEN WAS THE PROBLEM PRESENT:				
NOT AT ALL	A LITTLE BIT	MODER-ATELY	QUITE A BIT	EXTRE-MELY		ALL OF THE TIME	MOST OF THE TIME	SOME OF THE TIME	RARELY	NEVER
⓪	①	②	③	④	Feeling uncomfortable about eating or drinking in public	⓪	①	②	③	④
⓪	①	②	③	④	Getting into frequent arguments	⓪	①	②	③	④
⓪	①	②	③	④	Feeling nervous when you are left alone	⓪	①	②	③	④
⓪	①	②	③	④	Others not giving you proper credit for your	⓪	①	②	③	④
⓪	①	②	③	④	Feeling lonely even when you are with people	⓪	①	②	③	④
⓪	①	②	③	④	Feeling so restless you couldn't sit still	⓪	①	②	③	④
⓪	①	②	③	④	Feelings of worthlessness	⓪	①	②	③	④
⓪	①	②	③	④	The feeling that something bad is going to happen to you	⓪	①	②	③	④
⓪	①	②	③	④	Shouting or throwing things	⓪	①	②	③	④
⓪	①	②	③	④	Feeling afraid you will faint in public	⓪	①	②	③	④
⓪	①	②	③	④	Feeling that people will take advantage of you if you let them	⓪	①	②	③	④
⓪	①	②	③	④	Having thoughts about sex that bother you a lot	⓪	①	②	③	④
⓪	①	②	③	④	The idea that you should be punished for your sins	⓪	①	②	③	④
⓪	①	②	③	④	Thoughts or images of a frightening nature	⓪	①	②	③	④
⓪	①	②	③	④	The idea that something serious is wrong with your body	⓪	①	②	③	④
⓪	①	②	③	④	Never feeling close to another person	⓪	①	②	③	④
⓪	①	②	③	④	Feelings of guilt	⓪	①	②	③	④
⓪	①	②	③	④	The idea that something is wrong with your mind	⓪	①	②	③	④
⓪	①	②	③	④	Abdominal pain / Bleeding	⓪	①	②	③	④
⓪	①	②	③	④	Allergies	⓪	①	②	③	④
⓪	①	②	③	④	Constipation	⓪	①	②	③	④
⓪	①	②	③	④	Depression	⓪	①	②	③	④
⓪	①	②	③	④	Diarrhoea	⓪	①	②	③	④
⓪	①	②	③	④	Anxiety	⓪	①	②	③	④
⓪	①	②	③	④	Bladder infections / Cystitis	⓪	①	②	③	④
⓪	①	②	③	④	Blurry vision	⓪	①	②	③	④
⓪	①	②	③	④	Burning urination (Passing water)	⓪	①	②	③	④
⓪	①	②	③	④	Confusion	⓪	①	②	③	④

HOW MUCH WERE YOU DISTRESSED BY:						HOW OFTEN WAS THE PROBLEM PRESENT:				
NOT AT ALL	A LITTLE BIT	MODER-ATELY	QUITE A BIT	EXTRE-MELY	PROBLEM	ALL OF THE TIME	MOST OF THE TIME	SOME OF THE TIME	RARELY	NEVER
⓪	①	②	③	④	Recurrent genital infections	⓪	①	②	③	④
⓪	①	②	③	④	Ringing in the ears	⓪	①	②	③	④
⓪	①	②	③	④	Sore glands	⓪	①	②	③	④
⓪	①	②	③	④	Dry eyes	⓪	①	②	③	④
⓪	①	②	③	④	Jaw joint pain or discomfort	⓪	①	②	③	④
⓪	①	②	③	④	Fatigue	⓪	①	②	③	④
⓪	①	②	③	④	Fear of closed spaces	⓪	①	②	③	④
⓪	①	②	③	④	Feverishness / nightsweats	⓪	①	②	③	④
⓪	①	②	③	④	Frequent urination	⓪	①	②	③	④
⓪	①	②	③	④	Frustration	⓪	①	②	③	④
⓪	①	②	③	④	Hair loss	⓪	①	②	③	④
⓪	①	②	③	④	Heartburn / Gastric reflux	⓪	①	②	③	④
⓪	①	②	③	④	Joint pain	⓪	①	②	③	④
⓪	①	②	③	④	Leg cramps	⓪	①	②	③	④
⓪	①	②	③	④	Muscle twitches	⓪	①	②	③	④
⓪	①	②	③	④	Nightmares	⓪	①	②	③	④
⓪	①	②	③	④	Paranoia	⓪	①	②	③	④
⓪	①	②	③	④	Poor circulation / cold hands and feet	⓪	①	②	③	④
⓪	①	②	③	④	Rashes	⓪	①	②	③	④
⓪	①	②	③	④	Sore throat	⓪	①	②	③	④
⓪	①	②	③	④	Stomach cramps	⓪	①	②	③	④
⓪	①	②	③	④	Earaches	⓪	①	②	③	④
⓪	①	②	③	④	Facial pain or discomfort	⓪	①	②	③	④
⓪	①	②	③	④	Swallowing difficulties	⓪	①	②	③	④
⓪	①	②	③	④	Tired eyes	⓪	①	②	③	④
⓪	①	②	③	④	Tooth grinding	⓪	①	②	③	④
⓪	①	②	③	④	Fingers / toes blueish colour sensitive to touch	⓪	①	②	③	④

29. Please answer the following question by marking the horizontal line with a straight vertical stroke (|) as shown in the example below:

Example:

Overall Strongly DISAGREE ______|____________________________________ **Overall Strongly AGREE**

"My state of health has made me different from people my age or in my 'well' peer group"

Overall Strongly DISAGREE ______|____________________________________ **Overall Strongly AGREE**

30. Please tick and comment **ONLY if you feel you are different from others your age or your 'well' peer group,** in any of the following areas. (You can write over the page if you feel you need more space to write your comments). **Please explain your answer.**

1) **Personality** **Yes** ☐ **No** ☐

If yes
☐ Overall the difference is negative
☐ Overall the difference is positive
☐ Overall the difference is both positive and negative

Please explain ____
____
____
____

2) **Maturity** **Yes** ☐ **No** ☐

If yes
☐ Overall the difference is negative
☐ Overall the difference is positive
☐ Overall the difference is both positive and negative

Please explain ____
____
____
____

3) **Outlook on Life** **Yes** ☐ **No** ☐

If yes
☐ Overall the difference is negative
☐ Overall the difference is positive
☐ Overall the difference is both positive and negative

Please explain ____
____
____
____

4) **Physical capabilities and health** **Yes** ☐ **No** ☐

If yes
☐ Overall the difference is negative
☐ Overall the difference is positive
☐ Overall the difference is both positive and negative

Please explain ____
____
____
____

5) **Social Relationships (Friendships / going out)** **Yes** ☐ **No** ☐

If yes
☐ Overall the difference is negative
☐ Overall the difference is positive
☐ Overall the difference is both positive and negative

Please explain ____
____
____
____

6) **Dating (Boyfriend / Girlfriend / Partner)** **Yes** ☐ **No** ☐

If yes
☐ Overall the difference is negative
☐ Overall the difference is positive
☐ Overall the difference is both positive and negative

Please explain ____
____
____
____

7) **School work / Achievement / Study** **Yes** ☐ **No** ☐

If yes
☐ Overall the difference is negative
☐ Overall the difference is positive
☐ Overall the difference is both positive and negative

Please explain ____
____
____
____

8) **In your relationship with your family** **Yes** ☐ **No** ☐

If yes
☐ Overall the difference is negative
☐ Overall the difference is positive
☐ Overall the difference is both positive and negative

Please explain ____
____
____
____

9) **In making plans for the future** **Yes** ☐ **No** ☐

If yes
☐ Overall the difference is negative
☐ Overall the difference is positive
☐ Overall the difference is both positive and negative

Please explain ____
____
____
____

10) **How you feel emotionally** **Yes** ☐ **No** ☐

If yes
☐ Overall the difference is negative
☐ Overall the difference is positive
☐ Overall the difference is both positive and negative

Please explain ____
____
____
____

11) **How you feel about your appearance and body (Body Image)** **Yes** ☐ **No** ☐

If yes
☐ Overall the difference is negative
☐ Overall the difference is positive
☐ Overall the difference is both positive and negative

Please explain ____
____
____
____

12) **In how independent you are** **Yes** ☐ **No** ☐

If yes
☐ Overall the difference is negative
☐ Overall the difference is positive
☐ Overall the difference is both positive and negative

Please explain ____
____
____
____

13) **How you feel about your sexuality/sexual activity** **Yes** ☐ **No** ☐

If yes
☐ Overall the difference is negative
☐ Overall the difference is positive
☐ Overall the difference is both positive and negative

Please explain ____

____

____

____

14) **In your ability to get / keep paid work** **Yes** ☐ **No** ☐

If yes
☐ Overall the difference is negative
☐ Overall the difference is positive
☐ Overall the difference is both positive and negative

Please explain ____

____

____

____

31. Please tick any event(s) that occurred in the last 5-8 years :

1) ☐ Tooth / Gum / Mouth Infection
2) ☐ Dental Treatment / Orthodontics
3) ☐ Q Fever
4) ☐ Blood Transfusion
5) ☐ Bacterial Infection
6) ☐ Hepatitis
7) ☐ Ross River Fever
8) ☐ Chest infection
9) ☐ Glandular Fever
10) ☐ Viral Infection
11) ☐ Bowel / tummy upset
12) ☐ Food poisoning
13) ☐ General anaesthetic
14) ☐ Flu / Cold
15) ☐ Vaccination
16) ☐ Urinary tract infection
17) ☐ Tick bite
18) ☐ Lice bite
19) ☐ Sandfly bite

20) ☐ Mosquito bite

21) ☐ Wasp sting

22) ☐ Bee sting

23) ☐ Spider sting

24) ☐ Hospital Stay

25) ☐ Medical treatment, please specify

____

26) ☐ Prolonged Stress

27) ☐ Stressful event, please specify

____

28) ☐ Exercise / Competitive Sport

29) ☐ Your house sprayed for pests/insects

30) ☐ Your neighbours house sprayed for pests/insects.

Exposure to:

31) ☐ Crop Spray

32) ☐ Chlorine

33) ☐ Pesticides

34) ☐ Defoliant

35) ☐ DDT

36) ☐ Paint Stripper

37) ☐ Acetone / Nail Polish Remover

38) ☐ Other, please specify

____

32. To the best of your knowledge have either of your parents been exposed to or have worked with any of the following :

1) ☐ Crop Dusting
2) ☐ Agent Orange
3) ☐ Leather Tanning Agents
4) ☐ Panel Beating Industry
5) ☐ Paint Industry
6) ☐ Chemical Industry
7) ☐ Cleaning Industry

33. Use a vertical stroke (|) to mark the horizontal lines as seen in the example below

Example

Not at all ____________________|______________________ **Severe reaction**

Consider how you react to the following :

1) ☐ **Extremes in temperature**

Not at all ____ **Severe reaction**

2) ☐ **Bright light**

Not at all ____ **Severe reaction**

3) ☐ **Loud noise**

Not at all ____ **Severe reaction**

4) ☐ **Acetone / Nail Polish Remover**

Not at all ____ **Severe reaction**

5) ☐ **Pesticide**

Not at all ____ **Severe reaction**

6) ☐ **Perfume**

Not at all ____ **Severe reaction**

7) ☐ **Glue**

Not at all ____ **Severe reaction**

8) ☐ **Texters / Pens / Liquidpaper / Whiteout**

Not at all ____ **Severe reaction**

9) ☐ **Strong Smells / Fumes**

Not at all ____ **Severe reaction**

10) ☐ **Ammonia**

Not at all ____ **Severe reaction**

11) ☐ **Other, (please specify)** ___________________________________

Not at all ____ **Severe reaction**

34. Have you ever taken, (including during childhood) antibiotics continuously for four weeks or longer?

☐ Yes
☐ No

35. Have you ever taken multiple courses of antibiotics ?

☐ Yes
☐ No

36.a) Have you ever reacted badly to any medications ?

☐ Yes
☐ **No, go to Question 37**

36.b) If yes, to which ones ? ______________________________

____

How did you react ? ______________________________

____

____

When did you react ? eg. (straight away, 12 hours later etc.) ______

____

____

37. Do any other members of your family have C.F.S., R.R.V, Clinical Depression ?

CFS ☐ Yes ☐ No   RRV ☐ Yes ☐ No   Clinical Depression ☐ Yes ☐ No

If yes, which members (eg. sister, aunt ***no names***) ?

CFS: ______________________________

RRV: ______________________________

Clinical Depression: ______________________________

38.a) Do any of your close friends, <u>not including MEYA or Support Group friends</u> have C.F.S. R.R.V Clinical Depression ?

☐ Yes

☐ **No, go to Question 39**

☐ Female Friend

☐ Male Friend

☐ Intimate Friend

39. How many teeth do you have Amalgam fillings in?

☐ **No Amalgam fillings, go to Question 43**

☐ 1-4 fillings

☐ 5-9 fillings

☐ More than 9 fillings

40. Have you had any of your Amalgam fillings removed?

☐ Yes
☐ **No, go to Question 43**

If yes, why?

____

____

41. Did the removal of the fillings help?

☐ Yes
☐ No

42. How long ago did you have the fillings removed?

________ Years ________ Months

43. Did you need assistance to fill out this questionnaire?

☐ Yes
☐ **No, go to Question 45**

If yes, who gave you assistance to fill it out? ______________________

44. Approximately how long did it take you to fill out this questionnaire?

____

THANK YOU !!!

If you have any further comments, please write below and over the page

Seven-day Symptom Questionnaire

SCL-90-R®

Symptom Checklist-90-R

Leonard R. Derogatis, PhD

Last Name First MI

ID Number

Age Gender Test Date / /

DIRECTIONS

1. Print your name, identification number, age, gender, and testing date in the area on this page.
2. Use a lead pencil only and make a dark mark when responding to the items on pages 2 and 3.
3. If you want to change an answer, erase it carefully and then fill in your new choice
4. Do not make any marks outside the circles.

DO NOT SEND TO NATIONAL COMPUTER SYSTEMS
USE ONLY FOR HAND SCORING

INSTRUCTIONS

Below is a list of problems people sometimes have. Please read each on carefully, and blacken the circle that best describes HOW MUCH THAT PROBLEM HAS DISTRESSED OR BOTHERED YOU DURING THE PAST 7 DAYS INCLUDING TODAY.

Blacken the circle for only one number for each problem and do not skip any items. If you change your mind, erase your first mark carefully. Read the example before beginning, and if you have any questions please ask about them.

	NOT AT ALL	A LITTLE BIT	MODER-ATELY	QUITE A BIT	EXTRE-MELY	EXAMPLE HOW MUCH WERE YOU DISTRESSED BY:
66	⓪	①	②	●	④	Bodyaches

	NOT AT ALL	A LITTLE BIT	MODER-ATELY	QUITE A BIT	EXTRE-MELY	HOW MUCH WERE YOU DISTRESSED BY:
1	⓪	①	②	③	④	Headaches
2	⓪	①	②	③	④	Nervousness or shakiness inside
3	⓪	①	②	③	④	Unpleasant thoughts that won't leave your mind
4	⓪	①	②	③	④	Faintness or dizziness
5	⓪	①	②	③	④	Loss of sexual interest or pleasure
6	⓪	①	②	③	④	Feeling critical of others
7	⓪	①	②	③	④	The idea that someone else can control your thoughts
8	⓪	①	②	③	④	Feeling that others are to blame for most of your
9	⓪	①	②	③	④	Trouble remembering things
10	⓪	①	②	③	④	Worried about sloppiness or carelessness
11	⓪	①	②	③	④	Feeling easily annoyed or irritated
12	⓪	①	②	③	④	Pains in heart or chest
13	⓪	①	②	③	④	Feeling afraid in open spaces or on the streets
14	⓪	①	②	③	④	Feeling low in energy or slowed down
15	⓪	①	②	③	④	Thoughts of ending your life
16	⓪	①	②	③	④	Hearing voices that other people do not hear
17	⓪	①	②	③	④	Trembling
18	⓪	①	②	③	④	Feeling that most people cannot be trusted
19	⓪	①	②	③	④	Poor appetite
20	⓪	①	②	③	④	Crying easily
21	⓪	①	②	③	④	Feeling shy or uneasy with the opposite sex
22	⓪	①	②	③	④	Feelings of being trapped or caught
23	⓪	①	②	③	④	Suddenly scared for no reason
24	⓪	①	②	③	④	Temper outbursts that you cannot control
25	⓪	①	②	③	④	Feeling afraid to go out of your house alone
26	⓪	①	②	③	④	Blaming yourself for things
27	⓪	①	②	③	④	Pains in the lower back
28	⓪	①	②	③	④	Feeling blocked in getting things done
29	⓪	①	②	③	④	Feeling lonely
30	⓪	①	②	③	④	Feeling blue
31	⓪	①	②	③	④	Worrying too much about things
32	⓪	①	②	③	④	Feeling no interest in things
33	⓪	①	②	③	④	Feeling fearful
34	⓪	①	②	③	④	Your feelings being easily hurt
35	⓪	①	②	③	④	Other people being aware of your private thoughts
36	⓪	①	②	③	④	Feeling others don't understand you or are
37	⓪	①	②	③	④	Feeling that people are unfriendly or dislike you
38	⓪	①	②	③	④	Having to do things very slowly to ensure correctness
39	⓪	①	②	③	④	Heart pounding or racing

	NOT AT ALL	A LITTLE BIT	MODER-ATELY	QUITE A BIT	EXTRE-MELY	HOW MUCH WERE YOU DISTRESSED BY:
40	⓪	①	②	③	④	Nausea or upset stomach
41	⓪	①	②	③	④	Feeling inferior to others
42	⓪	①	②	③	④	Soreness of your muscles
43	⓪	①	②	③	④	Feeling that you are watched or talked about by others
44	⓪	①	②	③	④	Trouble falling asleep
45	⓪	①	②	③	④	Having to check and double-check what you do
46	⓪	①	②	③	④	Difficulty making decisions
47	⓪	①	②	③	④	Feeling afraid to travel on buses, subways or trains
48	⓪	①	②	③	④	Trouble getting your breath
49	⓪	①	②	③	④	Hot or cold spells
50	⓪	①	②	③	④	Having to avoid certain things, places, or activities
51	⓪	①	②	③	④	Your mind going blank
52	⓪	①	②	③	④	Numbness or tingling in parts of your body
53	⓪	①	②	③	④	A lump in your throat
54	⓪	①	②	③	④	Felling hopelessness about the future
55	⓪	①	②	③	④	Trouble concentrating
56	⓪	①	②	③	④	Feeling weak in parts of your body
57	⓪	①	②	③	④	Feeling tense or keyed up
58	⓪	①	②	③	④	Heavy feelings in your arms or legs
59	⓪	①	②	③	④	Thoughts of death or dying
60	⓪	①	②	③	④	Overeating
61	⓪	①	②	③	④	Feeling uneasy when people are watching or talking
62	⓪	①	②	③	④	Having thoughts that are not your own
63	⓪	①	②	③	④	Having urges to beat, injure or harm someone
64	⓪	①	②	③	④	Awakening in the early morning
65	⓪	①	②	③	④	Having to repeat the same actions such as touching,
66	⓪	①	②	③	④	Sleep that is restless or disturbed
67	⓪	①	②	③	④	Having urges to break or smash things
68	⓪	①	②	③	④	Having ideas or beliefs that others do not share
69	⓪	①	②	③	④	Feeling very self-conscious with others
70	⓪	①	②	③	④	Feeling uneasy in crowds, such as shopping or at a
71	⓪	①	②	③	④	Feeling everything is an effort
72	⓪	①	②	③	④	Spells of terror or panic
73	⓪	①	②	③	④	Feeling uncomfortable about eating or drinking in
74	⓪	①	②	③	④	Getting into frequent arguments
75	⓪	①	②	③	④	Feeling nervous when you are left alone
76	⓪	①	②	③	④	Others not giving you proper credit for your
77	⓪	①	②	③	④	Feeling lonely even when you are with people
78	⓪	①	②	③	④	Feeling so restless you couldn't sit still
79	⓪	①	②	③	④	Feeling of worthlessness
80	⓪	①	②	③	④	The feeling that something bad is going to happen to
81	⓪	①	②	③	④	Shouting or throwing things
82	⓪	①	②	③	④	Feeling afraid you will faint in public
83	⓪	①	②	③	④	Feeling that people will take advantage of you if you let
84	⓪	①	②	③	④	Having thoughts about sex that bother you a lot
85	⓪	①	②	③	④	The idea that you should be punished for your sins
86	⓪	①	②	③	④	Thoughts or images of a frightening nature
87	⓪	①	②	③	④	The idea that something serious is wrong with your
88	⓪	①	②	③	④	Never feeling close to another person
89	⓪	①	②	③	④	Feelings of guilt
90	⓪	①	②	③	④	The idea that something is wrong with your mind

INSTRUCTIONS

Below is a list of problems people sometimes have. Please read each on carefully, and blacken the circle that best describes HOW MUCH THAT PROBLEM HAS DISTRESSED OR BOTHERED YOU DURING THE PAST 7 DAYS INCLUDING TODAY.

Blacken the circle for only one number for each problem and do not skip any items. If you change your mind, erase your first mark carefully. Read the example before beginning, and if you have any questions please ask about them.

NOT AT ALL	A LITTLE BIT	MODER-ATELY	QUITE A BIT	EXTRE-MELY	EXAMPLE HOW MUCH WERE YOU DISTRESSED BY:
⓪	①	②	●	④	Bodyaches

NOT AT ALL	A LITTLE BIT	MODER-ATELY	QUITE A BIT	EXTRE-MELY	HOW MUCH WERE YOU DISTRESSED BY:
⓪	①	②	③	④	Abdominal pain / Bleeding
⓪	①	②	③	④	Allergies
⓪	①	②	③	④	Constipation
⓪	①	②	③	④	Depression
⓪	①	②	③	④	Diarrhoea
⓪	①	②	③	④	Anxiety
⓪	①	②	③	④	Bladder infections / Cystitis
⓪	①	②	③	④	Blurry vision
⓪	①	②	③	④	Burning urination (Passing water)
⓪	①	②	③	④	Confusion
⓪	①	②	③	④	Recurrent genital infections
⓪	①	②	③	④	Ringing in the ears
⓪	①	②	③	④	Sore glands
⓪	①	②	③	④	Dry eyes
⓪	①	②	③	④	Jaw joint pain or discomfort
⓪	①	②	③	④	Fatigue
⓪	①	②	③	④	Fear of closed spaces
⓪	①	②	③	④	Feverishness / Nightsweats
⓪	①	②	③	④	Frequent urination
⓪	①	②	③	④	Frustration
⓪	①	②	③	④	Hair loss
⓪	①	②	③	④	Heartburn / Gastric reflux
⓪	①	②	③	④	Joint pain
⓪	①	②	③	④	Leg cramps
⓪	①	②	③	④	Muscle twitches
⓪	①	②	③	④	Nightmares
⓪	①	②	③	④	Paranoia
⓪	①	②	③	④	Poor circulation / cold hands and feet
⓪	①	②	③	④	Rashes
⓪	①	②	③	④	Sore throat
⓪	①	②	③	④	Stomach cramps
⓪	①	②	③	④	Earaches
⓪	①	②	③	④	Facial pain or discomfort
⓪	①	②	③	④	Swallowing difficulties
⓪	①	②	③	④	Tired eyes
⓪	①	②	③	④	Tooth grinding
⓪	①	②	③	④	Fingers / toes blueish colour sensitive to touch

What is the most accurate representation of your situation in the last 7 days?

- ☐ Completely bedbound; unable to leave the house.
- ☐ Housebound, occasional outings.
- ☐ Large periods of time resting, out once a week.
- ☐ Minimal activities out once a week.
- ☐ Reduced activities eg. school/ uni/ work, out some days.
- ☐ Slightly reduced activity level, eg. school / uni / work most days.
- ☐ Completely well.

COGNITIVE FAILURES QUESTIONNAIRE.

The following questions are about minor mistakes which everyone makes from time to time, but some of which happen more often than others. We want to know how often these things have happened to you in the last six months. Please circle the appropriate number.

	Very Often	Quite Often	Occasionally	Very Rarely	Never
1. Do you read something and find you haven't been thinking about it and must read it again ?	4	3	2	1	0
2. Do you find you forget why you went from one part of the house to the other ?	4	3	2	1	0
3. Do you fail to notice signposts on the road ?	4	3	2	1	0
4. Do you find you confuse right and left when giving directions ?	4	3	2	1	0
5. Do you bump into people ?	4	3	2	1	0
6. Do you find that you forget whether you have turned off a light or locked the door ?	4	3	2	1	0
7. Do you fail to listen to peoples names when you meet them ?	4	3	2	1	0
8. Do you say something and realize afterwards that it might be taken as insulting ?	4	3	2	1	0
9. Do you fail to hear people speaking to you when you are doing something else ?	4	3	2	1	0
10. Do you lose your temper and regret it ?	4	3	2	1	0
11. Do you leave important letters unanswered for days ?	4	3	2	1	0
12. Do you find you forget which way to turn on a road you know well, but rarely use ?	4	3	2	1	0
13. Do you fail to see what you want in a supermarket, (even though it's there)	4	3	2	1	0
14. Do you find yourself wondering whether you've used a word correctly ?	4	3	2	1	0
15. Do you have trouble making up your mind ?	4	3	2	1	0
16. Do you find you forget appointments ?	4	3	2	1	0
17. Do you forget where you put something like a newspaper or a book ?	4	3	2	1	0
18. Do you find you accidentally throw away the thing you want, and keep the thing you meant to throw away- as in the example of throwing away the matchbox and putting the match in your pocket ?	4	3	2	1	0

	Very Often	Quite Often	Occasionally	Very Rarely	Never
19. Do you daydream when you ought to be listening to something ?	4	3	2	1	0
20. Do you find you forget peoples names ?	4	3	2	1	0
21. Do you start doing something at home, and get distracted doing something else ? (unintentionally)	4	3	2	1	0
22. Do you find you cant quite remember something, although it is on the tip of your tongue ?	4	3	2	1	0
23. Do you find you forget what you came to the shops to buy ?	4	3	2	1	0
24. Do you drop things ?	4	3	2	1	0
25. Do you find you cant think of anything to say ?	4	3	2	1	0

CPRU Questionnaire

APPENDIX IV

PLEASE CIRCLE THE NUMBER THAT BEST DESCRIBES YOUR RESPONSE.

Name: .... **Date**: ...../...../.....

Internal use only
Receiving Date: ...../...../.....
CU Ref Number: CU...........

Referring Doctor: ....

Scores: 0 = *Not at all*; 1 = *a little bit*, 2 = *moderately*; 3 = *quite a bit*; 4 = *extremely.*

IN THE LAST 7 DAYS HOW MUCH WERE YOU AFFECTED BY:		0	1	2	3	4
HEADACHES	*1*	0	1	2	3	4
NERVOUS OR SHAKINESS INSIDE	*2*	0	1	2	3	4
REPEATED UNPLEASANT THOUGHTS ABOUT YOUR PROBLEMS	*3*	0	1	2	3	4
FAINTNESS OR DIZZINESS	*4*	0	1	2	3	4
LOSS OF LIBIDO OR SEXUAL INTEREST	*5*	0	1	2	3	4
UNUSUAL SWEATING WHILST ASLEEP (NIGHT SWEATS)	*6*	0	1	2	3	4
MIGRAINE HEAD ACHES	*7*	0	1	2	3	4
UNUSUAL MUSCLE TWITCHES	*8*	0	1	2	3	4
TROUBLE REMEMBERING THINGS	*9*	0	1	2	3	4
FREQUENT MUSCLE CRAMPS	*10*	0	1	2	3	4
GRINDING OR CLENCHING YOUR TEETH	*11*	0	1	2	3	4
CHEST OR HEART PAIN	*12*	0	1	2	3	4
FACE PAIN OR TENDERNESS	*13*	0	1	2	3	4
FEELING LOW IN ENERGY OR SLOWED DOWN	*14*	0	1	2	3	4
NECK PAIN OR TENDERNESS	*15*	0	1	2	3	4
SHOULDER PAIN OR TENDERNESS	*16*	0	1	2	3	4
TREMBLING	*17*	0	1	2	3	4
ARTHRITIS	*18*	0	1	2	3	4
POOR APPETITE	*19*	0	1	2	3	4
CRYING EASILY OVER YOUR PROBLEMS	*20*	0	1	2	3	4
ARM PAIN OR TENDERNESS	*21*	0	1	2	3	4
LEG PAIN OR TENDERNESS	*22*	0	1	2	3	4
ABDOMINAL PAIN OR TENDERNESS	*23*	0	1	2	3	4
PAINFUL OR STIFF JOINTS UPON AWAKENING IN THE MORNING	*24*	0	1	2	3	4
JOINTS THAT HURT WITH MOVEMENT	*25*	0	1	2	3	4
LOCKING OR CLICKING JAW JOINT	*26*	0	1	2	3	4
LOW BACK PAIN OR TENDERNESS	*27*	0	1	2	3	4
FEELING YOUR PROBLEMS ARE DISTURBING YOUR NORMAL LIFE	*28*	0	1	2	3	4
TINNITUS OR NOISES IN THE EAR	*29*	0	1	2	3	4
FEELING BLUE AS A RESULT OF YOUR PROBLEMS	*30*	0	1	2	3	4
SORE THROATS OR NASAL INFECTIONS	*31*	0	1	2	3	4
FEELING NO INTEREST IN THINGS	*32*	0	1	2	3	4
DISLIKE OF STRONG LIGHT OR PHOTOPHOBIA	*33*	0	1	2	3	4
UNREFRESHED OR PROLONGED SLEEP	*34*	0	1	2	3	4
STRESS FROM FINANCIAL PROBLEMS	*35*	0	1	2	3	4
FEELING OTHERS ARE UNSYMPATHETIC ABOUT YOUR PROBLEMS	*36*	0	1	2	3	4
UNEXPLAINED DIARRHOEA	*37*	0	1	2	3	4
HAVING TO DO THINGS SLOWLY TO ENSURE CORRECTNESS	*38*	0	1	2	3	4
HEART POUNDING, RACING OR PALPITATIONS	*39*	0	1	2	3	4
NAUSEA OR UPSET STOMACH	*40*	0	1	2	3	4
CONSTIPATION	*41*	0	1	2	3	4
MUSCLE SORENESS OR STIFFNESS	*42*	0	1	2	3	4

PLEASE TURN OVER

CPRU Questionnaire

Scores: 0 = *Not at all*; 1 = *a little bit*, 2 = *moderately*; 3 = *quite a bit;* 4 = *extremely.*

IN THE LAST 7 DAYS HOW MUCH WERE YOU AFFECTED BY:		0	1	2	3	4
FREQUENT URINATION	*43*	0	1	2	3	4
TROUBLE FALLING ASLEEP	*44*	0	1	2	3	4
HAVING TO CHECK AND DOUBLE CHECK WHAT YOU ARE DOING	*45*	0	1	2	3	4
DIFFICULTY IN MAKING DECISIONS	*46*	0	1	2	3	4
OVULATION OR MENSTRUAL PAIN	*47*	0	1	2	3	4
EXERTIONAL CHEST PAIN OR BREATHLESSNESS	*48*	0	1	2	3	4
HOT AND COLD SPELLS OR RECURRENT FEVERISHNESS	*49*	0	1	2	3	4
AVOIDING CERTAIN ACTIVITIES DUE TO PHYSICAL PROBLEMS	*50*	0	1	2	3	4
MIND GOING BLANK	*51*	0	1	2	3	4
LOSS OF FEELING, TINGLING OR NUMBNESS OF THE SKIN	*52*	0	1	2	3	4
SORE OR SWOLLEN LYMPH GLANDS IN YOUR NECK	*53*	0	1	2	3	4
FEELING HOPELESS ABOUT THE FUTURE	*54*	0	1	2	3	4
TROUBLE CONCENTRATING	*55*	0	1	2	3	4
MUSCLE WEAKNESS OR WEAK FEELINGS IN THE BODY	*56*	0	1	2	3	4
BURNING OR UNCOMFORTABLE URINATION	*57*	0	1	2	3	4
HEAVY FEELINGS IN THE LIMBS	*58*	0	1	2	3	4
SORE OR SWOLLEN LYMPH GLANDS UNDER YOUR ARMS	*59*	0	1	2	3	4
ORCHALGIA OR TESTICULAR PAIN	*60*	0	1	2	3	4
ALLERGIES	*61*	0	1	2	3	4
FORGETFULNESS	*62*	0	1	2	3	4
URGENT URINATION	*63*	0	1	2	3	4
TROUBLE AWAKENING IN THE MORNING	*64*	0	1	2	3	4
SORE OR SWOLLEN LYMPH GLANDS IN YOUR GROIN	*65*	0	1	2	3	4
SLEEP THAT IS RESTLESS OR DISTURBED	*66*	0	1	2	3	4
VAGINAL IRRITATION OR DISCOMFORT	*67*	0	1	2	3	4
HYPERSENSITIVITY OF THE SKIN	*68*	0	1	2	3	4
FEELINGS OF MENTAL TIREDNESS OR FATIGUE	*69*	0	1	2	3	4
DIFFICULTY USING WORDS OR LANGUAGE	*70*	0	1	2	3	4
FEELING THAT EVERYTHING IS AN EFFORT	*71*	0	1	2	3	4
SPELLS OF PANIC RELATED TO YOUR PROBLEMS	*72*	0	1	2	3	4
SCIATICA	*73*	0	1	2	3	4
GETTING INTO FREQUENT ARGUMENTS	*74*	0	1	2	3	4
FEELING NERVOUS ABOUT YOUR PROBLEMS WHEN LEFT ALONE	*75*	0	1	2	3	4
RECURRENT ORAL ULCERATION	*76*	0	1	2	3	4
SYMPTOMS OF IRRITABLE BOWEL	*77*	0	1	2	3	4
MENTAL CONFUSION OR LOSS OF YOUR TRAIN OF THOUGHT	*78*	0	1	2	3	4
STRESSFUL EVENTS IN YOUR LIFE RELATED TO YOUR PROBLEMS	*79*	0	1	2	3	4
DERMATITIS	*80*	0	1	2	3	4
STRESS OVER FAMILY PROBLEMS	*81*	0	1	2	3	4
GASTRIC REFLUX OR HEARTBURN	*82*	0	1	2	3	4
CRAVINGS FOR CERTAIN FOODS	*83*	0	1	2	3	4
BLOOD PRESSURE PROBLEMS	*84*	0	1	2	3	4
STRESS FROM WORK PROBLEMS	*85*	0	1	2	3	4
THE IDEA SOMETHING IS SERIOUSLY WRONG WITH YOUR BODY	*86*	0	1	2	3	4