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.

![Graph showing Total Metabolite Excreted over Duration (years).](image)

**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.

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

![Diagram of pain response phases](image)

**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 opioids 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 potentiates 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 hyponatreemic 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; Vimpani 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.

3.7. References.


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