CHAPTER 5.


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).

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

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.](image)

**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 opioids 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. Adrenocorticotropic 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 E₂ 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 PGE2 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, IL-6) 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 = Adrenocorticotropic 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 alteration 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 dopaminergic 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 was 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](image_url)

**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).
Blood Glucose

Liver

Inhibited reaction

Gluconeogenesis

ALT Reaction

Enhanced reaction

Glycolysis

ALT Reaction

Blood alanine

Glutamine

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*

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* 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, χ²=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.

5.5. References.
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