3.4. RESULTS.

3.4.1. RDC/TMD type 1a study.

3.4.1.a. Patient Details.

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

3.4.1.b. Urinary Excretion Patterns.

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

Tyrosine was higher in relative abundance in the RDC/TMD type 1a group and the tyrosine: leucine ratio was elevated in the RDC/TMD type 1a patients compared with the control subjects (RDC/TMD type 1a=7.21±1.91, C=1.13±0.23; $P<0.00004$). Thus the tyrosine: leucine ratio, the marker ratio of dysregulation proteolysis: protein synthesis, was strongly associated with facial pain.
Table 3.1. T-test analysis of the Urine Excretion Data Measured in the RDC/TMD type 1a Patients and Control Subjects.

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Control</th>
<th>RDC/TMD type 1a</th>
<th>P</th>
<th>RA Control</th>
<th>RA MP</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Peak area(SE)</td>
<td>mmoles(SE)</td>
<td>Peak area(SE)</td>
<td>mmoles(SE)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 Ethanolamine</td>
<td>21.85(3.07)</td>
<td>2.70(0.03)</td>
<td>20.15(2.74)</td>
<td>2.69(0.03)</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>2 Serine</td>
<td>79.94(13.67)</td>
<td>0.87(0.05)</td>
<td>63.83(9.46)</td>
<td>0.81(0.03)</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>3 Alanine</td>
<td>55.84(7.97)</td>
<td>1.80(0.08)</td>
<td>41.77(6.14)</td>
<td>1.65(0.06)</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>4 Glycine</td>
<td>219.39(25.79)</td>
<td>1.98(0.11)</td>
<td>184.56(25.32)</td>
<td>1.84(0.10)</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>5 β-Alanine</td>
<td>7.69(1.82)</td>
<td>1.02(0.01)</td>
<td>11.30(2.52)</td>
<td>1.04(0.01)</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>5a Valine</td>
<td>11.03(2.09)</td>
<td>0.12(0.01)</td>
<td>8.68(1.17)</td>
<td>0.11(0.00)</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>5b Threonine</td>
<td>19.25(3.80)</td>
<td>1.23(0.01)</td>
<td>15.50(2.13)</td>
<td>1.22(0.01)</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>6 β-Aminoisobutyrate</td>
<td>5.99(1.23)</td>
<td>-</td>
<td>8.50(2.07)</td>
<td>-</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>7 Leucine</td>
<td>4.26(1.19)</td>
<td>0.11(0.00)</td>
<td>0.80(0.31)</td>
<td>0.10(0.00)</td>
<td>&lt;1.0E-&lt;5</td>
<td>&lt;1.0E-&lt;5</td>
</tr>
<tr>
<td>8 Phenylacetic acid</td>
<td>19.14(2.96)</td>
<td>-</td>
<td>23.07(4.35)</td>
<td>-</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>9 Prolene</td>
<td>22.91(3.86)</td>
<td>0.59(0.01)</td>
<td>18.88(2.71)</td>
<td>0.58(0.01)</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>10 Succinic acid</td>
<td>15.85(2.66)</td>
<td>6.15(0.61)</td>
<td>21.63(4.52)</td>
<td>7.47(1.04)</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>10a S-Methylcystiene</td>
<td>3.48(0.53)</td>
<td>0.80(0.01)</td>
<td>5.21(1.24)</td>
<td>0.83(0.02)</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>11 Asparagine</td>
<td>16.18(1.91)</td>
<td>0.17(0.02)</td>
<td>19.73(2.70)</td>
<td>0.21(0.03)</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>11a Hydroxyproline</td>
<td>6.33(1.15)</td>
<td>0.72(0.00)</td>
<td>5.44(0.87)</td>
<td>0.72(0.00)</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>12 CFSUM1</td>
<td>30.92(7.52)</td>
<td>-</td>
<td>71.20(14.43)</td>
<td>-</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>13 UM13</td>
<td>0.54(0.19)</td>
<td>-</td>
<td>0.32(0.08)</td>
<td>-</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>13a UM13a</td>
<td>1.71(0.41)</td>
<td>-</td>
<td>3.26(1.78)</td>
<td>-</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>14 UM14</td>
<td>1.47(0.49)</td>
<td>-</td>
<td>1.17(0.26)</td>
<td>-</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>15 UM15</td>
<td>0.15(0.08)</td>
<td>-</td>
<td>0.53(0.38)</td>
<td>-</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>15a UM15a</td>
<td>5.12(0.83)</td>
<td>-</td>
<td>8.52(2.15)</td>
<td>-</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>15b Acetaminophen</td>
<td>0.10(0.08)</td>
<td>-</td>
<td>6.06(3.36)</td>
<td>&lt;0.03</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>16 Aspartic acid</td>
<td>22.95(4.64)</td>
<td>0.22(0.02)</td>
<td>23.68(3.69)</td>
<td>0.23(0.01)</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>17 UM17</td>
<td>1.47(0.31)</td>
<td>-</td>
<td>1.83(0.50)</td>
<td>-</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>18 Phenylalanine</td>
<td>11.93(1.80)</td>
<td>0.41(0.00)</td>
<td>13.27(2.01)</td>
<td>0.41(0.00)</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>19 Ornithine</td>
<td>10.61(2.10)</td>
<td>1.56(0.00)</td>
<td>11.71(3.09)</td>
<td>1.56(0.00)</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>20 Glutamic acid</td>
<td>141.70(27.69)</td>
<td>0.99(0.10)</td>
<td>124.10(14.48)</td>
<td>0.93(0.05)</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>21 Lysine</td>
<td>58.07(16.99)</td>
<td>1.31(0.01)</td>
<td>55.56(16.96)</td>
<td>1.31(0.01)</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>22 Tyrosine</td>
<td>4.71(1.24)</td>
<td>2.87(0.00)</td>
<td>7.65(1.75)</td>
<td>2.87(0.00)</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>23 1-Methylhistidine</td>
<td>18.32(11.61)</td>
<td>0.32(0.01)</td>
<td>37.14(20.95)</td>
<td>0.33(0.01)</td>
<td>&lt;0.03</td>
<td>&lt;0.03</td>
</tr>
<tr>
<td>24 3-Methylhistidine</td>
<td>20.43(4.28)</td>
<td>0.02(0.01)</td>
<td>21.35(4.38)</td>
<td>0.03(0.01)</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>25 Hippuric acid</td>
<td>286.32(61.35)</td>
<td>1.46(0.27)</td>
<td>373.41(87.13)</td>
<td>1.83(0.38)</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>26 Acetonic acid</td>
<td>76.03(9.65)</td>
<td>0.43(0.03)</td>
<td>96.47(11.50)</td>
<td>0.48(0.03)</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>26a Citric acid</td>
<td>24.59(11.76)</td>
<td>5.80(0.24)</td>
<td>50.57(21.23)</td>
<td>6.32(0.43)</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>27 UM27</td>
<td>23.44(9.49)</td>
<td>-</td>
<td>24.35(6.26)</td>
<td>-</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>28 UM28</td>
<td>13.72(8.26)</td>
<td>-</td>
<td>11.52(2.25)</td>
<td>-</td>
<td>&lt;5.3E-4</td>
<td>&lt;5.3E-4</td>
</tr>
</tbody>
</table>

Total: 1263.4(172.1) 1392.7(150.7) NS
Figure 3.6 shows the scatter plot of the association between the tyrosine: leucine ratio and the symptom prevalence (the cumulative total of the positive responses to the SCL-90-R pain marker questions - Q1-headaches, Q4-faintness and dizziness, Q12-chest pain, Q14-low in energy or run down, Q27-low back pain, Q39-heart palpitations, Q40-nausea, Q42-muscle soreness, Q52-numbness or tingling, Q55-trouble concentrating, Q56-weakness, Q58-heavy feelings in limbs, Q66-restless or disturbed sleep).

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

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

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

Forward stepwise discriminant function established a difference in the urinary concentration excretion of amino and organic acids between males and females (Wilks' $\lambda = 0.610$, F=2.667, $P<0.008$). The first discriminate variable was unknown UM17 ($P<0.13$), followed by leucine ($P<0.13$), glycine ($P<0.09$) and phenylacetic acid ($P<0.11$).
Student t-test did not find any difference in any urinary parameter (concentration or relative abundance). Thus the differences between males and females is not large but involve alteration in the metabolism of leucine, glycine, UM17, UM15a, $\beta$-alanine and phenylacetic acid, as well as increased consumption of acetaminophen.

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

The RDC/TMD type 1a group had a mean VAS score of $2.8\pm1.0$ (±SD), as distinct from the control group which had a zero VAS score. Multiple regression analysis was applied to determine whether the increases in pain severity scores observed in the RDC/TMD type 1a group were directly correlated with changes in the urine excretion profiles. Within the RDC/TMD type 1a group, the primary correlate with increasing pain severity scores in the regression models were the reductions in the concentration and relative abundance of excreted leucine (Table 3.2). Table 3.2 also shows a summary of both the concentration and relative abundance regression and correlation analyses of the urinary metabolites for all study subjects and specifically for the RDC/TMD type 1a patients.

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

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

1) excretion of the non-fibrillar catabolism control amino acids (serine, glycine, valine, proline, phenylalanine, and glutamic acid), but not the fibrillar catabolism indicator, 3-methylhistidine;

2) tyrosine: leucine ratio suggesting dysregulation of proteolysis: protein synthesis;

3) total excretion of both amino and organic acids;
4) excretion of the phospholipid polar head-group amino acids, serine, ethanolamine; and
5) excretion of the excitatory amino acids, glutamic and aspartic acid.

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

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

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

**Concentration** regression model: $R^2=0.999$, $F=4705$, $P<0.02$,
Variables: 1) aspartic acid $+P<0.02$, 2) leucine $-P<0.009$, 3) acetaminophen $+P<0.03$.

**Relative abundance**, regression model: $R^2=0.848$, $F=4.450$, $P<0.007$,
Variables: 1) leucine $-P<0.0002$, 2) serine $-P<0.95$, 3) citric acid $+P<0.02$.

The mmolar and concentration values were identical and will not shown for subsequent analyses.
Figure 3.7 Scatter plot of the tyrosine: leucine ratio and its association with pain severity in all RDC/TMD type 1a study patients.

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

Figure 3.9 Scatter plot of pain severity and its association with aspartic acid excretion in the RDC/TMD type 1a study subjects.
3.4.1.e. Differences in Urinary Metabolites in Relationship to Symptom Prevalence.

The RDC/TMD type 1a group had an increase in the reporting of pain/fatigue symptoms (C = 2.1±1.7; RDC/TMD = 6.6±2.0 – \( P<2.0\times10^{-12} \)). Multiple regression analysis was applied to determine whether the increases in symptom prevalence were directly correlated with changes in urine excretion profiles. Within the RDC/TMD type 1a group, the primary correlate with increasing symptom prevalence, was the reduction in concentration and relative abundance of leucine (Table 3.3). Table 3.3 also shows a summary of the concentration and relative abundance regression and correlation analyses of the urinary metabolites of all study subjects and within the RDC/TMD type 1a patients. The association between the tyrosine: leucine ratio and symptom prevalence is shown in Figure 3.6 and was similar to that seen with pain severity.

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

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Concentration</th>
<th>Relative Abundance</th>
</tr>
</thead>
<tbody>
<tr>
<td>All study subjects</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tyrosine: leucine ratio</td>
<td>0.33</td>
<td>&lt;0.008</td>
</tr>
<tr>
<td>Total amino acid excretion</td>
<td>0.12</td>
<td>NS</td>
</tr>
<tr>
<td>Increased</td>
<td></td>
<td></td>
</tr>
<tr>
<td>UM28</td>
<td>0.43</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Acetaminophen</td>
<td>0.38</td>
<td>&lt;0.002</td>
</tr>
<tr>
<td>CFSUM1</td>
<td>0.27</td>
<td>&lt;0.04</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>0.20</td>
<td>NS</td>
</tr>
<tr>
<td>Decreased</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leucine</td>
<td>-0.53</td>
<td>&lt;7.0E-6</td>
</tr>
<tr>
<td>Alanine</td>
<td>-0.19</td>
<td>NS</td>
</tr>
<tr>
<td>Glycine</td>
<td>-0.12</td>
<td>NS</td>
</tr>
<tr>
<td>Serine</td>
<td>-0.09</td>
<td>NS</td>
</tr>
<tr>
<td>Valine</td>
<td>-0.14</td>
<td>NS</td>
</tr>
<tr>
<td>Threonine</td>
<td>-0.08</td>
<td>NS</td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>-0.03</td>
<td>NS</td>
</tr>
<tr>
<td>MP patients only</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glutamate</td>
<td>-0.13</td>
<td>NS</td>
</tr>
</tbody>
</table>

**Concentration** regression model: \( R^2=0.626, F=5.742, P<0.00000 \)
Variables: 1) leucine \( -P<0.00001 \), 2) S-Methylcysteine +\( P<0.002 \), 3) Acetaminophen +\( P<0.005 \).

**Relative abundance**, regression model: \( R^2=0.673, F=8.570, P<0.00000 \)
Variables: 1) leucine \( -P<0.000001 \), 2) Acetaminophen +\( P<0.008 \), 3) S-Methylcysteine +\( P<0.02 \).

Thus symptom severity was associated with alterations in the:

1) excretion of the non-fibrillar catabolism control amino acids (serine, glycine, valine, leucine, and glutamic acid);

2) tyrosine: leucine ratio suggesting dysregulation of proteolysis: protein synthesis; and

3) the muscle liver nitrogen exchange amino acids, alanine and glutamic acid.
These changes indicate an increase in both protein turnover and reductions in the availability of muscle produced nitrogen transfer amino acids.

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

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

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

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Concentration</th>
<th>Relative Abundance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>r</td>
<td>P</td>
</tr>
<tr>
<td>Increased</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3-Methylhistidine</td>
<td>0.26</td>
<td>NS</td>
</tr>
<tr>
<td>UM27</td>
<td>0.05</td>
<td>NS</td>
</tr>
<tr>
<td>Decreased</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Succinic acid</td>
<td>-0.58</td>
<td>&lt;0.003</td>
</tr>
<tr>
<td>Hippuric acid</td>
<td>-0.52</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Serine</td>
<td>-0.46</td>
<td>&lt;0.03</td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>-0.46</td>
<td>&lt;0.03</td>
</tr>
<tr>
<td>Phenylacetic acid</td>
<td>-0.45</td>
<td>&lt;0.03</td>
</tr>
<tr>
<td>Asparagine</td>
<td>-0.42</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Aspartic acid</td>
<td>-0.41</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

Concentration regression model: R²=0.879, F=9.486, P<0.0002
Variables: 1) succinic acid -P<0.006, 2) leucine -P<0.09, 3) UM17 +P<0.11.

Relative abundance regression model: R²=0.999, F=584600, P<0.001
Variables: 1) 3-methylhistidine +P<0.18, 2) aconitic acid -P<0.27, 3) UM13 +P<0.30.

The increase in duration of the illness was associated with quite different metabolite changes to that seen with pain severity and symptom prevalence. Illness duration was negatively associated with total amino acid excretion. In the RDC/TMD type 1a group, the relative abundance of 3-methylhistidine was negatively correlated with the total metabolite (r=-0.62, P<0.001), and positively correlated with the relative abundance of both leucine (r=-0.47, P<0.01) and tyrosine (r=0.46, P<0.01). However, in the control subjects, the relative abundance of 3-methylhistidine was not correlated with the total metabolite (r=-0.06) or the relative abundance of leucine (r=-0.11) or tyrosine (r=0.06).
These data show that as the total amino acid excretion falls with increasing duration of the illness, there is a corresponding increase in the excretion of the fibrillar catabolism marker, 3-methylhistidine in the RDC/TMD type 1a group. This increase in 3-methylhistidine is associated with increases in the relative abundance of both tyrosine and leucine, indicating a corresponding increase in protein synthesis, non-fibrillar and fibrillar proteolysis. Thus with increasing illness duration there was a progressive reduction in amino acid excretion, which was negatively associated with increases in marker metabolites suggesting increases in fibrillar and non-fibrillar proteolysis and protein synthesis.
3.4.1.g. Differences in Urinary Metabolites in Relationship to Sudden or Gradual Onset.

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

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

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Concentration</th>
<th>Relative Abundance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sudden</td>
<td>Gradual</td>
</tr>
<tr>
<td>Total metabolite tyrosine: leucine ratio</td>
<td>1575±1009</td>
<td>1409±720</td>
</tr>
<tr>
<td>Increased</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hydroxyproline</td>
<td>9.19±5.69</td>
<td>4.23±3.19</td>
</tr>
<tr>
<td>UM13a</td>
<td>8.13±16.62</td>
<td>1.32±1.61</td>
</tr>
<tr>
<td>Decreased</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Succinic acid</td>
<td>11.5±9.9</td>
<td>22.2±17.5</td>
</tr>
</tbody>
</table>

Concentration regression model: R²=0.990, F=27.358, P<0.0009
Variables: 1) Hydroxyproline +P<0.0003, 2) threonine -P<0.34, 3) 1-methylhistidine +P<0.0004.
Relative Abundance regression model: R²=0.999, F=187.13, P<0.0006
Variables: 1) Hydroxyproline +P<0.00003, 2) β-aminoisobutyrate -P<0.07, 3) Lysine -P<0.0001.

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

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

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

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Concentration</th>
<th>Relative Abundance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>r</td>
<td>P</td>
</tr>
<tr>
<td>All study subjects</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tyrosine: leucine ratio</td>
<td>0.60</td>
<td>&lt;0.00001</td>
</tr>
<tr>
<td>Total amino acid excretion</td>
<td>0.30</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Increased</td>
<td></td>
<td></td>
</tr>
<tr>
<td>UM28</td>
<td>0.64</td>
<td>&lt;0.000002</td>
</tr>
<tr>
<td>1-Methylhistidine</td>
<td>0.45</td>
<td>&lt;0.003</td>
</tr>
<tr>
<td>UM27</td>
<td>0.43</td>
<td>&lt;0.004</td>
</tr>
<tr>
<td>CFSUM1</td>
<td>0.42</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>Citric acid</td>
<td>0.37</td>
<td>&lt;0.02</td>
</tr>
<tr>
<td>β-Alanine</td>
<td>0.32</td>
<td>&lt;0.04</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>0.31</td>
<td>&lt;0.04</td>
</tr>
<tr>
<td>Decreased</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leucine</td>
<td>-0.54</td>
<td>&lt;0.0002</td>
</tr>
<tr>
<td>Glycine</td>
<td>-0.06</td>
<td>NS</td>
</tr>
<tr>
<td>Valine</td>
<td>-0.01</td>
<td>NS</td>
</tr>
<tr>
<td>Serine</td>
<td>0.06</td>
<td>NS</td>
</tr>
</tbody>
</table>

Concentration regression model: \( R^2=0.844, F=14.413, P<0.00000 \)
Variables: 1) UM28 +P<0.000002, 2) phenylacetic acid -P<0.003, 3) leucine -P<0.12.

Relative abundance regression model: \( R^2=0.804, F=12.308, P<0.00000 \)
Variables: 1) UM28 +P<0.000001, 2) 1-methylhistidine +P<0.005, 3) leucine -P<0.004.
The prime discriminate variable for increasing body pain distribution (all subjects) was UM28 and was different from that of pain severity, however increasing body pain distribution was positively associated with the tyrosine: leucine ratio and total amino acids excreted as seen with increasing pain severity. Thus increasing body pain distribution is similar to pain severity when assessing all study subjects, but is not associated with any urinary amino or organic acid alteration within the RDC/TMD type 1a pain group.

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

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

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

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Concentration</th>
<th>Relative Abundance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$r$</td>
<td>$P$</td>
</tr>
<tr>
<td><strong>All study subjects</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tyrosine: Leucine ratio</td>
<td>0.35</td>
<td>&lt;0.02</td>
</tr>
<tr>
<td><strong>Increased</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Citric acid</td>
<td>0.37</td>
<td>&lt;0.009</td>
</tr>
<tr>
<td>UM28</td>
<td>0.26</td>
<td>NS</td>
</tr>
<tr>
<td><strong>Decreased</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leucine</td>
<td>-0.31</td>
<td>&lt;0.03</td>
</tr>
<tr>
<td>Glycine</td>
<td>-0.17</td>
<td>NS</td>
</tr>
<tr>
<td>Serine</td>
<td>-0.18</td>
<td>NS</td>
</tr>
<tr>
<td><strong>MP patients only</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Increased</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Citric acid</td>
<td>0.54</td>
<td>&lt;0.03</td>
</tr>
<tr>
<td>Decreased</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glycine</td>
<td>-0.30</td>
<td>NS</td>
</tr>
</tbody>
</table>

**Concentration** regression model: $R^2=0.649, F=3.883, P<0.0001$
Variables: 1) leucine $+P<0.002$, 2) $+UM28 -P<0.04$, 3) UM15 $-P<0.06$.

**Relative abundance** regression model: $R^2=0.609, F=5.342, P<0.00001$
Variables: 1) serine $-P<0.0007$, 2) aspartic acid $+P<0.006$, 3) acetaminophen $+P<0.07$. 
3.4.1.j. Differences in Urinary Metabolites in Relationship to Fatigue.

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

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

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>All study subjects</th>
<th>MP patients only</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Concentration</td>
<td>Relative Abundance</td>
</tr>
<tr>
<td></td>
<td>$r$ $P$</td>
<td>$r$ $P$</td>
</tr>
<tr>
<td>Increased</td>
<td></td>
<td></td>
</tr>
<tr>
<td>UM28</td>
<td>0.34 &lt;0.02</td>
<td>0.35 &lt;0.02</td>
</tr>
<tr>
<td>3-Methylhistidine</td>
<td>0.20 NS</td>
<td>0.35 &lt;0.02</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>0.23 NS</td>
<td>0.32 &lt;0.03</td>
</tr>
<tr>
<td>UM27</td>
<td>0.20 NS</td>
<td>0.33 &lt;0.03</td>
</tr>
<tr>
<td>Decreased</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leucine</td>
<td>-0.41 &lt;0.004</td>
<td>-0.39 &lt;0.006</td>
</tr>
<tr>
<td>Succinic acid</td>
<td>-0.11 NS</td>
<td>-0.34 &lt;0.02</td>
</tr>
<tr>
<td>Glycine</td>
<td>-0.11 NS</td>
<td>-0.33 &lt;0.03</td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>-0.05 NS</td>
<td>-0.30 &lt;0.04</td>
</tr>
<tr>
<td>Phenylacetic acid</td>
<td>-0.12 NS</td>
<td>-0.29 &lt;0.04</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Increased</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3-Methylhistidine</td>
<td>0.43 NS</td>
<td>0.52 &lt;0.04</td>
</tr>
<tr>
<td>Decreased</td>
<td></td>
<td></td>
</tr>
<tr>
<td>UM13a</td>
<td>-0.56 &lt;0.02</td>
<td>-0.68 &lt;0.003</td>
</tr>
<tr>
<td>Succinic acid</td>
<td>-0.45 NS</td>
<td>-0.50 &lt;0.04</td>
</tr>
</tbody>
</table>

**Concentration** regression model: $R^2=0.633$, $F=6.553$, $P<0.00001$
Variables: 1) leucine -$P<0.0003$, 2) 3-methylhistidine $P<0.0003$, 3) UM14 $P<0.005$.

**Relative abundance** regression model: $R^2=0.707$, $F=7.237$, $P<0.00000$
Variables: 1) leucine -$P<0.00003$, 2) phenylacetic acid -$P<0.005$, 3) 3-methylhistidine $P<0.003$.

Fatigue was a primary symptom for determining the difference between fibromyalgia-like RDC/TMD type 1a patients and the remaining RDC/TMD type 1a patients, and was associated with increasing body pain distribution and symptom prevalence. These data show that whilst muscle soreness and fatigue are common symptoms in RDC/TMD type 1a patients, they are related to different metabolites which shows that they are associated with different biochemical events.
3.4.1.k. Differences in Urinary Metabolites in Relationship to SCL-90-R Somatization.

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

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

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Concentration</th>
<th>Relative Abundance</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Somatization Dimension</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All study subjects</td>
<td>$r$</td>
<td>$P$</td>
</tr>
<tr>
<td>Tyrosine: leucine ratio</td>
<td>0.37</td>
<td>$&lt;0.01$</td>
</tr>
<tr>
<td>Increased</td>
<td></td>
<td></td>
</tr>
<tr>
<td>UM28</td>
<td>0.41</td>
<td>$&lt;0.004$</td>
</tr>
<tr>
<td>CFSUM1</td>
<td>0.32</td>
<td>$&lt;0.03$</td>
</tr>
<tr>
<td>Citric acid</td>
<td>0.27</td>
<td>NS</td>
</tr>
<tr>
<td>Decreased</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leucine</td>
<td>-0.31</td>
<td>$&lt;0.04$</td>
</tr>
<tr>
<td>UM13a</td>
<td>-0.18</td>
<td>NS</td>
</tr>
<tr>
<td>Glycine</td>
<td>-0.07</td>
<td>NS</td>
</tr>
</tbody>
</table>

**Concentration regression model:** $R^2=0.520, F=3.255, P<0.003$
Variables: 1) 3-methylhistidine $P<0.04$, 2) leucine $-P<0.008$, 3) UM13a $-P<0.07$.

**Relative abundance regression model:** $R^2=0.527, F=3.347, P<0.003$
Variables: 1) glycine $-P<0.006$, 2) UM13a $-P<0.04$, 3) 3-methylhistidine $P<0.03$.

| **Depression Dimension** | | |
| All study subjects | $r$ | $P$ | $r$ | $P$ |
| Increased | | |
| Citric acid | 0.27 | NS | 0.30 | $<0.04$ |
| Decreased | | |
| Glycine | -0.09 | NS | -0.33 | $<0.02$ |

**Concentration regression model:** $R^2=0.702, F=3.926, P<0.0005$
Variables: 1) 3-methylhistidine $P<0.06$, 2) threonine $-P<0.03$, 3) citric acid $P<0.08$.

**Relative abundance regression model:** $R^2=0.523, F=2.666, P<0.01$
Variables: 1) glycine $-P<0.01$, 2) 3-methylhistidine $-P<0.21$, 3) citric acid $P<0.11$. 
3.4.1.l. Differences in Urinary Metabolites in Relationship to SCL-90-R Depression.

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

3.4.2. TMD Pain in CFS Study.

3.4.2.a. Patient Characteristics.

A total of 100 CFS patients were recruited and 83 age- and sex-matched control subjects were recruited as presented in Chapter 2. There was no difference in any characteristics when comparing the 100 CFS patients \((\text{age} = 29.1\pm12.5\ \text{years}; \ \text{range} \ 13-73\ \text{years}; \ % \text{female} \ 73.0)\) with the 83 control subjects \((\text{age} = 32.8\pm13.9\ \text{years}; \ \text{range} \ 14-69\ \text{years}; \ % \text{female} \ 72.3)\).

3.4.2.b. Prevalence of TMD Symptoms.

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

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

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

3.4.2.d. Urine Amino and Organic Acids.

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

The tyrosine: leucine ratio was 2.32±5.15 in the control group and 3.09±4.28 in the CFS group. Twenty-nine control subjects did not report any muscle pain over the previous 12-months and this group had a mean tyrosine: leucine ratio of 1.26 (Standard Error = 0.34, Standard Deviation =1.82) that was very similar to the pain free RDC/TMD type 1a control group (Mean = 1.13, Standard Error = 0.23, Standard Deviation =1.21). Forty-eight CFS patients had a muscle pain score >2 and their mean tyrosine: leucine ratio was 4.00 (Standard Error = 0.78, Standard Deviation =5.39) which was less than the mean tyrosine: leucine ratio of the RDC/TMD type 1a group (Mean = 7.21, Standard Error = 1.91, Standard Deviation
=6.62). These data show that in patients with muscle pain (both the RDC/TMD and CFS study groups) that the 2 Standard Deviation range for the tyrosine: leucine ratio was between 3.55 and 4.9, a mean value of 4.2.

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

<table>
<thead>
<tr>
<th>Variable</th>
<th>Excretion rate/minute</th>
<th>Relative Abundance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control (n=81) X (SE)</td>
<td>CFS (n=96) X (SE)</td>
</tr>
<tr>
<td>Tyrosine: leucine</td>
<td>2.32(5.15)</td>
<td>3.09(4.28)</td>
</tr>
<tr>
<td>Increased</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acetaminophen</td>
<td>2.5 (1.1)</td>
<td>77.0 (32.3)</td>
</tr>
<tr>
<td>3-Methylhistidine</td>
<td>84.8 (11.5)</td>
<td>107.7 (13.9)</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>24.9 (3.8)</td>
<td>30.4 (3.8)</td>
</tr>
<tr>
<td>UM27</td>
<td>67.3 (12.7)</td>
<td>85.1 (13.3)</td>
</tr>
<tr>
<td>1-Methylhistidine</td>
<td>91.1 (24.2)</td>
<td>145.2 (32.1)</td>
</tr>
<tr>
<td>UM28</td>
<td>4.6 (0.8)</td>
<td>6.2 (1.0)</td>
</tr>
<tr>
<td>Decreased</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Asparagine</td>
<td>57.6 (4.8)</td>
<td>35.7 (3.5)</td>
</tr>
<tr>
<td>Succinic acid</td>
<td>34.3 (2.9)</td>
<td>23.7 (2.5)</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>36.5 (2.8)</td>
<td>28.0 (2.6)</td>
</tr>
<tr>
<td>UM17</td>
<td>5.0 (0.5)</td>
<td>3.4 (0.4)</td>
</tr>
<tr>
<td>Hippuric acid</td>
<td>1009.1 (124.0)</td>
<td>807.2 (130.4)</td>
</tr>
<tr>
<td>Proline</td>
<td>74.1 (6.9)</td>
<td>56.8 (3.4)</td>
</tr>
<tr>
<td>Alanine</td>
<td>236.2 (16.8)</td>
<td>193.1 (14.8)</td>
</tr>
<tr>
<td>Valine</td>
<td>37.0 (2.4)</td>
<td>31.0 (2.0)</td>
</tr>
<tr>
<td>Leucine</td>
<td>14.7 (1.0)</td>
<td>12.1 (0.8)</td>
</tr>
<tr>
<td>S-Methylcysteine</td>
<td>10.7 (1.2)</td>
<td>7.8 (0.9)</td>
</tr>
</tbody>
</table>

Excretion rate/minute regression model: $R^2=0.318$, $F=4.724$, $P<0.00000$
Variables: 1) asparagine -$P<0.00007$, 2) acetaminophen $P<0.04$, 3) 3-methylhistidine $P<0.06$.

Relative abundance regression model: $R^2=0.279$, $F=3.970$, $P<0.00000$
Variables: 1) asparagine -$P<0.002$, 2) hippuric acid -$P<0.009$, 3) acetaminophen -$P<0.04$.

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

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

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

<table>
<thead>
<tr>
<th>Serum Parameter</th>
<th>mL/Minute</th>
<th>Metabolite/Minute</th>
<th>Organic acids/Minute</th>
<th>Amino acids/Minute</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urine</td>
<td></td>
<td>r P</td>
<td>r P</td>
<td>r P</td>
</tr>
<tr>
<td>Tyrosine: leucine ratio</td>
<td>0.28 &lt;0.007</td>
<td>0.25 &lt;0.02</td>
<td>0.23 &lt;0.03</td>
<td>0.26 &lt;0.02</td>
</tr>
</tbody>
</table>

Excretion rate/minute

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

**mL/minute** regression model; R²=0.376, F=6.623, P<0.00000
Variables; 1) Glutamic acid P<0.0007; 2) Citric acid P<0.002, 3) lysine P<0.00005.

**Metabolite/minute** regression model; R²=0.922, F=54.998, P<0.00000
Variables; 1) Aspartic acid P<0.006, 2) Hippuric acid P<3.0E-11, 3) Glycine P<0.00002.

**Amino acids/minute** regression model; R²=0.976, F=145.76, P<0.00000
Variables; 1) Threonine P<0.005, 2) Glycine P<1.0E-14, 3) Lysine P<0.009

**Organic acids/minute** regression model; R²=0.914, F=43.156, P<0.00000
Variables; 1) Hippuric acid P<1.0E-14, 2) Aspartic acid P<0.03, 3) Citric acid P<0.11.

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

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

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

**ml/minute** regression model; R²=0.465, F=4.347, P<0.00000
Variables; 1) Lysine P<0.005, 2) UM27 P<0.87, 3) Serine -P<0.00003.

**Metabolite/minute** regression model; R²=0.695, F=10.600, P<0.00000
Variables; 1) Proline -P<0.04, 2) Lysine P<4.6E-8, 3) Glutamic acid P<0.81

**Amino acids/minute** regression model; R²=0.713, F=10.072, P<0.00000
Variables; 1) Lysine P<1.4E-13, 2) Glycine P<0.000002, 3) Proline -P<0.008.

**Organic acids/minute** regression model; R²=0.808, F=17.092, P<0.00000
Variables; 1) Proline -P<0.02, 2) Hippuric acid P<3.0E-8, 3) Citric acid P<1.5E7.
3.4.2.f. Serum Amino and Organic Acids.

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

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

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

Standard serum biochemistry was obtained from the CFS patients but not the control subjects and therefore could not be assessed (Table 3.14). However none of the variables were considered to be outside the normal 2 standard deviation range nor indicative of pathology.
Table 3.13. Multiple regression and t-test analysis of the changes in serum metabolites between CFS patients and control subjects.

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Excretion rate/ minute</th>
<th>Relative Abundance</th>
<th>Control</th>
<th>CFS</th>
<th>P</th>
<th>Control</th>
<th>CFS</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>X (SE)</td>
<td>X (SE)</td>
<td></td>
<td>X (SE)</td>
<td>X (SE)</td>
<td></td>
</tr>
<tr>
<td>Tyrosine: leucine</td>
<td></td>
<td></td>
<td>0.43</td>
<td>0.34</td>
<td>&lt;0.00009</td>
<td></td>
<td>0.54</td>
<td>0.34</td>
</tr>
<tr>
<td>Increased</td>
<td>Ph2a</td>
<td>7023(749)</td>
<td>0.34(0.07)</td>
<td>12231(743)</td>
<td>&lt;2.0E-8</td>
<td>0.54(0.06)</td>
<td>1.06(0.06)</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td></td>
<td>Ethanolamine</td>
<td>6985(474)</td>
<td>0.34(0.07)</td>
<td>8421(451)</td>
<td>&lt;0.005</td>
<td>0.54(0.04)</td>
<td>0.72(0.04)</td>
<td>&lt;0.03</td>
</tr>
<tr>
<td></td>
<td>Ph1</td>
<td>31543(1507)</td>
<td>0.34(0.07)</td>
<td>33959(2181)</td>
<td>&lt;0.005</td>
<td>2.35(0.11)</td>
<td>2.81(0.18)</td>
<td>NS</td>
</tr>
<tr>
<td>Decreased</td>
<td>Ph2a</td>
<td>64735(3008)</td>
<td>0.34(0.07)</td>
<td>53245(3167)</td>
<td>&lt;0.00002</td>
<td>4.56(0.15)</td>
<td>3.79(0.10)</td>
<td>&lt;0.00006</td>
</tr>
<tr>
<td></td>
<td>Tyrosine</td>
<td>35812(1563)</td>
<td>0.34(0.07)</td>
<td>27192(1785)</td>
<td>&lt;0.00004</td>
<td>2.54(0.09)</td>
<td>1.96(0.10)</td>
<td>&lt;0.00006</td>
</tr>
<tr>
<td></td>
<td>β-Aminoisobutyrate</td>
<td>12532(504)</td>
<td>0.34(0.07)</td>
<td>10282(494)</td>
<td>&lt;0.009</td>
<td>0.9(0.03)</td>
<td>0.80(0.03)</td>
<td>&lt;0.007</td>
</tr>
<tr>
<td></td>
<td>β-Alanine</td>
<td>1656(198)</td>
<td>0.34(0.07)</td>
<td>1149(94)</td>
<td>&lt;0.02</td>
<td>0.11(0.01)</td>
<td>0.08(0.01)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>No Change</td>
<td>Serine</td>
<td>40321(2212)</td>
<td>0.34(0.07)</td>
<td>40829(2409)</td>
<td>NS</td>
<td>2.92(0.13)</td>
<td>2.95(0.10)</td>
<td>NS</td>
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<tr>
<td></td>
<td>Alanine</td>
<td>174389(5271)</td>
<td>0.34(0.07)</td>
<td>169575(6260)</td>
<td>NS</td>
<td>12.84(0.29)</td>
<td>13.55(0.28)</td>
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<tr>
<td></td>
<td>Glycine</td>
<td>90763(3057)</td>
<td>0.34(0.07)</td>
<td>89494(4069)</td>
<td>NS</td>
<td>6.64(0.16)</td>
<td>6.90(0.18)</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>Valine</td>
<td>135092(3778)</td>
<td>0.34(0.07)</td>
<td>121282(4265)</td>
<td>NS</td>
<td>9.88(0.17)</td>
<td>9.77(0.21)</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>Threonine</td>
<td>82377(3410)</td>
<td>0.34(0.07)</td>
<td>73285(2986)</td>
<td>NS</td>
<td>5.82(0.21)</td>
<td>5.75(0.16)</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>Aminohydroxypropionate</td>
<td>19328(1556)</td>
<td>0.34(0.07)</td>
<td>18344(1540)</td>
<td>NS</td>
<td>1.45(0.12)</td>
<td>1.49(0.11)</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>Ph2</td>
<td>13209(1008)</td>
<td>0.34(0.07)</td>
<td>10874(688)</td>
<td>NS</td>
<td>0.98(0.07)</td>
<td>0.94(0.06)</td>
<td>NS</td>
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<tr>
<td></td>
<td>Leucine</td>
<td>83787(2551)</td>
<td>0.34(0.07)</td>
<td>73923(2876)</td>
<td>NS</td>
<td>6.10(0.12)</td>
<td>5.85(0.11)</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>Isoleucine</td>
<td>40628(1276)</td>
<td>0.34(0.07)</td>
<td>38172(1475)</td>
<td>NS</td>
<td>2.96(0.06)</td>
<td>3.00(0.06)</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>Proline</td>
<td>111755(6070)</td>
<td>0.34(0.07)</td>
<td>123649(7605)</td>
<td>NS</td>
<td>8.02(0.36)</td>
<td>8.81(0.37)</td>
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</tr>
<tr>
<td></td>
<td>S-methylcysteine</td>
<td>716(206)</td>
<td>0.34(0.07)</td>
<td>1188(262)</td>
<td>NS</td>
<td>0.05(0.01)</td>
<td>0.08(0.01)</td>
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<tr>
<td></td>
<td>Asparagine</td>
<td>4407(407)</td>
<td>0.34(0.07)</td>
<td>3734(345)</td>
<td>NS</td>
<td>0.32(0.03)</td>
<td>0.26(0.02)</td>
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<tr>
<td></td>
<td>Aspartic acid</td>
<td>45885(2583)</td>
<td>0.34(0.07)</td>
<td>42056(3143)</td>
<td>NS</td>
<td>3.25(0.13)</td>
<td>2.92(0.13)</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>Ornithine</td>
<td>66173(3872)</td>
<td>0.34(0.07)</td>
<td>58208(4004)</td>
<td>NS</td>
<td>4.73(0.28)</td>
<td>4.44(0.27)</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>Glutamic acid</td>
<td>252041(10451)</td>
<td>0.34(0.07)</td>
<td>241022(13850)</td>
<td>NS</td>
<td>18.77(0.44)</td>
<td>17.37(0.44)</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>Lysine</td>
<td>55308(3360)</td>
<td>0.34(0.07)</td>
<td>56132(3789)</td>
<td>NS</td>
<td>3.85(0.18)</td>
<td>4.04(0.17)</td>
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</tr>
<tr>
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<td>Aconitic acid</td>
<td>4798(388)</td>
<td>0.34(0.07)</td>
<td>5743(548)</td>
<td>NS</td>
<td>0.34(0.02)</td>
<td>0.40(0.03)</td>
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<td></td>
<td>Tryptophan</td>
<td>4215(450)</td>
<td>0.34(0.07)</td>
<td>3290(390)</td>
<td>NS</td>
<td>0.28(0.03)</td>
<td>0.22(0.02)</td>
<td>NS</td>
</tr>
</tbody>
</table>

Concentration regression model: \( R^2=0.272, F=10.773, P<0.00000 \); Variables: 1) Ph2a \( P<0.00001 \), 2) β-aminoisobutyrate \( -P<0.0005 \), 3) ornithine \( P<0.06 \).

Relative abundance regression model: \( R^2=0.312, F=7.650, P<0.00000 \); Variables: 1) Ph2a \( P<0.002 \), 2) β-aminoisobutyrate \( -P<0.003 \), 3) tyrosine \( -P<0.09 \).

<table>
<thead>
<tr>
<th>Variable</th>
<th>CFS X (S.D.)</th>
<th>Control X (S.D.)</th>
<th>P (t-test)</th>
<th>Prevalence CFS Abnormal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Red cell count (10^12/L)</td>
<td>4.61 (0.40)</td>
<td>4.62 (0.41)</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Haemoglobin (g/L)</td>
<td>13.85 (1.10)</td>
<td>13.69 (1.15)</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Haematocrit (L/L)</td>
<td>40.44 (3.19)</td>
<td>40.07 (3.37)</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Mean corpuscular volume (fL)</td>
<td>87.74 (3.16)</td>
<td>86.75 (3.77)</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Mean cell haemoglobin (pg)</td>
<td>30.07 (1.30)</td>
<td>29.68 (1.49)</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Mean cell haemoglobin conc. (g/100mL)</td>
<td>34.25 (0.62)</td>
<td>34.17 (0.59)</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Red cell distribution width (%)</td>
<td>12.35 (0.94)</td>
<td>12.58 (0.83)</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Platelet count (10^9/L)</td>
<td>253.1 (60.7)</td>
<td>256.1 (62.9)</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Mean platelet volume (fL)</td>
<td>8.60 (0.99)</td>
<td>8.36 (0.98)</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Leukocyte count (10^9/L)</td>
<td>6.11 (1.44)</td>
<td>5.74 (1.36)</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Lymphocyte count (10^9/L)</td>
<td>1.95 (0.56)</td>
<td>1.93 (0.60)</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Neutrophil count (10^9/L)</td>
<td>3.46 (1.10)</td>
<td>3.12 (0.84)</td>
<td>&lt;0.03</td>
<td>15 &lt;0.004</td>
</tr>
<tr>
<td>Neutrophil : Lymphocyte ratio</td>
<td>1.91 (0.80)</td>
<td>1.68 (0.49)</td>
<td>&lt;0.03</td>
<td>NS</td>
</tr>
<tr>
<td>Monocyte count (10^9/L)</td>
<td>0.48 (0.15)</td>
<td>0.47 (0.14)</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Eosinophil count (10^9/L)</td>
<td>0.19 (0.15)</td>
<td>0.20 (0.18)</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Basophil count (10^9/L)</td>
<td>0.02 (0.02)</td>
<td>0.02 (0.03)</td>
<td>NS</td>
<td>NS</td>
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<tr>
<td>% Neutrophils</td>
<td>56.17 (8.66)</td>
<td>54.07 (6.87)</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>% Lymphocytes</td>
<td>32.60 (8.02)</td>
<td>33.78 (6.22)</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>% Monocytes</td>
<td>7.86 (1.55)</td>
<td>8.36 (1.80)</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>% Eosinophils</td>
<td>3.08 (2.09)</td>
<td>3.36 (2.69)</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>% Basophils</td>
<td>0.33 (0.28)</td>
<td>0.38 (0.47)</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

Pesticides

<table>
<thead>
<tr>
<th>Pesticides</th>
<th>X(prev %)</th>
<th>Mean</th>
<th>Prevalence</th>
</tr>
</thead>
<tbody>
<tr>
<td>DDE</td>
<td>3.73 (96.9%)</td>
<td>2.98 (97.5%)</td>
<td>NS</td>
</tr>
<tr>
<td>HCB</td>
<td>0.24 (12.4%)</td>
<td>0.48 (27.5%)</td>
<td>&lt;0.03</td>
</tr>
<tr>
<td>Heptochlor</td>
<td>0.19 (11.3%)</td>
<td>0.05 (5.0%)</td>
<td>NS</td>
</tr>
<tr>
<td>Deidrin</td>
<td>0.01 (9.3%)</td>
<td>0.06 (5.0%)</td>
<td>NS</td>
</tr>
<tr>
<td>β-BHC</td>
<td>0.04 (5.2%)</td>
<td>0.11 (10.0%)</td>
<td>NS</td>
</tr>
<tr>
<td>Aldrin</td>
<td>0.04 (3.1%)</td>
<td>0.01 (1.3%)</td>
<td>NS</td>
</tr>
<tr>
<td>α-BHC</td>
<td>0.01 (1.0%)</td>
<td>0</td>
<td>NS</td>
</tr>
<tr>
<td>DDT</td>
<td>0</td>
<td>0</td>
<td>NS</td>
</tr>
</tbody>
</table>

Discriminant Function Analyses
Blood cell parameters: Wilks’ $\lambda = 0.801$, $F=1.868$, $P<0.02$
Pesticides: Wilks’ $\lambda = 0.943$, $F=2.592$, $P<0.04$

3.4.2.h. Pesticides.

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

3.4.2.i. Reported facial pain.

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

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

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Facial Pain</th>
<th>No Facial Pain</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Increased % or X(SD)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ANA</td>
<td>68%</td>
<td>25%</td>
<td>&lt;0.0005</td>
</tr>
<tr>
<td>ANA Titre</td>
<td>115.2(205)</td>
<td>17.8(42)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Mean corpuscular Volume (MCV)</td>
<td>89.1(2.9)</td>
<td>87.4(3.1)</td>
<td>&lt;0.02</td>
</tr>
<tr>
<td>Serum Ph1</td>
<td>37923(25091)</td>
<td>32758(20713)</td>
<td>&lt;0.02</td>
</tr>
<tr>
<td>% Urine Lysine</td>
<td>7.13(14.8)</td>
<td>2.73(2.71)</td>
<td>&lt;0.04</td>
</tr>
<tr>
<td>% Urine Aspartic acid</td>
<td>1.76(0.92)</td>
<td>1.39(0.58)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Decreased % or X(SD)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Proline</td>
<td>95228(70848)</td>
<td>133090(76263)</td>
<td>&lt;0.009</td>
</tr>
</tbody>
</table>

Facial pain regression model; $R^2=0.400$, $F=2.670$, $P<0.02$
Variables: 1) ANA titre $P<0.005$, 2) Serum Ph1 $P<0.09$, 3) ERPM Urinary Hippuric acid $P<0.14$.

3.4.2. 7-Day Facial Pain Severity.

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

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

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

<table>
<thead>
<tr>
<th>Serum Parameter</th>
<th>Facial Pain</th>
<th>No Facial Pain</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>T-test 7-day Facial pain positive v controls</strong></td>
<td>X(SE)</td>
<td>X(SE)</td>
</tr>
<tr>
<td>Increased</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serum Ph2a</td>
<td>10218(7344)</td>
<td>4433(3953)</td>
</tr>
<tr>
<td>Serum Ethanolamine</td>
<td>8451(3962)</td>
<td>5968(2708)</td>
</tr>
<tr>
<td>% Neutrophils</td>
<td>58.4(8.0)</td>
<td>52.4(8.3)</td>
</tr>
<tr>
<td>Urinary tyrosine: leucine ratio</td>
<td>2.32(1.23)</td>
<td>1.51(2.5)</td>
</tr>
<tr>
<td>% and ERPM Acetaminophen</td>
<td>0.91(2.38)</td>
<td>0 &lt;0.03</td>
</tr>
<tr>
<td>% and ERPM Tyrosine</td>
<td>0.78(0.65)</td>
<td>0.41(0.58) &lt;0.03</td>
</tr>
<tr>
<td>Decreased</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serum Phenylalanine</td>
<td>57231(30147)</td>
<td>65218(20369) &lt;0.05</td>
</tr>
<tr>
<td>Serum tyrosine: leucine ratio</td>
<td>0.37(0.16)</td>
<td>0.48(0.16) &lt;0.05</td>
</tr>
<tr>
<td>% UM17</td>
<td>0.08(0.05)</td>
<td>0.13(0.10) &lt;0.009</td>
</tr>
<tr>
<td>% UM15a</td>
<td>0.51(0.80)</td>
<td>0.44(0.30) &lt;0.03</td>
</tr>
<tr>
<td>ERPM Asparagine</td>
<td>53.6(86.4)</td>
<td>59.6(38.1) &lt;0.05</td>
</tr>
<tr>
<td><strong>Correlation analysis</strong></td>
<td>CFS</td>
<td>Control</td>
</tr>
<tr>
<td>Increased</td>
<td>r P</td>
<td></td>
</tr>
<tr>
<td>% Urine Glutamine/Glutamic acid</td>
<td>-0.09 NS</td>
<td>0.35 &lt;0.02</td>
</tr>
<tr>
<td>% Urine Serine</td>
<td>-0.01 NS</td>
<td>0.28 &lt;0.05</td>
</tr>
<tr>
<td>Urine volume</td>
<td>0.03 NS</td>
<td>0.28 &lt;0.05</td>
</tr>
<tr>
<td>Decreased</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sodium</td>
<td>-0.29 &lt;0.02</td>
<td>-</td>
</tr>
<tr>
<td>Chloride</td>
<td>-0.25 &lt;0.05</td>
<td>-</td>
</tr>
<tr>
<td>Haemoglobin</td>
<td>-0.25 &lt;0.05</td>
<td>-</td>
</tr>
<tr>
<td>% Urine 3-Methylhistidine</td>
<td>-0.25 &lt;0.05</td>
<td>0.11 NS</td>
</tr>
</tbody>
</table>

7-Day facial pain severity in CFS patients regression model; $R^2=0.483$, $F=2.708$, $P<0.02$
Variables; 1) Sodium $P<0.005$, 2) Serum glycine $P>0.14$, 3) Serum tyrosine $P<0.008$.

7-Day facial pain severity in Control subjects regression model; $R^2=0.941$, $F=16.665$, $P<0.00000$
Variables; 1) % U Glutamic acid $P<0.0004$, 2) Serum β-aminoisobutyrate $P<0.00003$, 3) DDE $P<0.00006$.

Multiple regression and univariate analyses (excluding the serum chemistry) were also applied to the 7-day facial pain severity response in the control subjects. The primary regression variable in the control subjects for 7-day facial pain was the % glutamic acid, followed by a reduction in serum β-aminoisobutyrate and an increase in the pesticide DDE. The 7-day response was associated with an increase in urine volume and increases in the relative abundance of the excitatory amino acid, glutamic acid and the phospholipid head-group amino acid, serine. These changes are very similar to those for pain severity in the RDC/TMD type 1a group.
3.4.2.k. 12-Month Facial Pain Severity.

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

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

<table>
<thead>
<tr>
<th>Correlation Analysis</th>
<th>CFS</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum Increased</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ALT</td>
<td>0.40 &lt;0.001</td>
<td>-</td>
</tr>
<tr>
<td>AST</td>
<td>0.36 &lt;0.001</td>
<td>-</td>
</tr>
<tr>
<td>Basophil count</td>
<td>0.29 &lt;0.008</td>
<td>-0.12 NS</td>
</tr>
<tr>
<td>% and ERPM Urinary Aspartic acid</td>
<td>0.28 &lt;0.02</td>
<td>-0.05 NS</td>
</tr>
<tr>
<td>Erythrocyte Sedimentation rate (ESR)</td>
<td>0.27 &lt;0.02</td>
<td>-</td>
</tr>
<tr>
<td>Protein</td>
<td>0.27 &lt;0.02</td>
<td>-</td>
</tr>
<tr>
<td>Urine volume</td>
<td>0.27 &lt;0.02</td>
<td>0.04 NS</td>
</tr>
<tr>
<td>Serum β-Alanine</td>
<td>0.24 &lt;0.03</td>
<td>0.06 NS</td>
</tr>
<tr>
<td>% and ERPM Urinary Acetaminophen</td>
<td>0.24 &lt;0.03</td>
<td>-0.09 NS</td>
</tr>
<tr>
<td>ERPM Urinary Glutamic acid</td>
<td>0.23 &lt;0.04</td>
<td>-0.05 NS</td>
</tr>
<tr>
<td>Neutrophil count</td>
<td>0.23 &lt;0.04</td>
<td>-0.11 NS</td>
</tr>
<tr>
<td>Leukocyte count</td>
<td>0.22 &lt;0.05</td>
<td>-0.17 NS</td>
</tr>
<tr>
<td>Serum Glutamic acid</td>
<td>0.22 &lt;0.04</td>
<td>0.19 NS</td>
</tr>
<tr>
<td>Pesticide residue DDE</td>
<td>0.22 &lt;0.05</td>
<td>0.16 NS</td>
</tr>
<tr>
<td>Decreased</td>
<td></td>
<td></td>
</tr>
<tr>
<td>% and ERPM Urinary Hippuric acid</td>
<td>-0.24 &lt;0.04</td>
<td>0.12 NS</td>
</tr>
<tr>
<td>Serum Isoleucine</td>
<td>-0.23 &lt;0.04</td>
<td>-0.08 NS</td>
</tr>
<tr>
<td>Serum Proline</td>
<td>-0.22 &lt;0.05</td>
<td>-0.08 NS</td>
</tr>
<tr>
<td>% Urinary UM27</td>
<td>-0.22 &lt;0.05</td>
<td>-0.11 NS</td>
</tr>
<tr>
<td>ERPM Urinary CFSUM1</td>
<td>-0.22 &lt;0.05</td>
<td>0.09 NS</td>
</tr>
<tr>
<td>ERPM Urinary UM28</td>
<td>-0.22 &lt;0.05</td>
<td>0.09 NS</td>
</tr>
<tr>
<td>Serum Ph1</td>
<td>0.07 NS</td>
<td>-0.29 &lt;0.02</td>
</tr>
<tr>
<td>Serum β-Aminoisobutyrate</td>
<td>0.13 NS</td>
<td>-0.26 &lt;0.03</td>
</tr>
</tbody>
</table>

12-month facial pain severity CFS patient regression model; R²=0.648, F=6.456, P<0.00001
Variables; 1) ALT P<0.28, 2) ESR P<0.007, 3) Serum iron P<0.009.

12-month facial pain severity Control subject regression model; R²=0.908, F=7.765, P<0.00000
Variables; 1) Serum Ph1-P<0.003, 2) % Eosinophils -P<0.003, 3) % urinary UM14 P<0.000000004.

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

3.4.2.1. 12-Month Facial Pain Frequency.

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

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

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

<table>
<thead>
<tr>
<th>Serum Parameter</th>
<th>Facial pain</th>
<th>No Facial Pain</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>T-Test Facial pain positive v remaining patients</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Increased</td>
<td></td>
<td></td>
</tr>
<tr>
<td>% and ERPM Urinary Acetaminophen</td>
<td>2.67%</td>
<td>0.26% &lt;0.001</td>
</tr>
<tr>
<td>Pesticide residue DDE</td>
<td>4.68</td>
<td>2.78 &lt;0.005</td>
</tr>
<tr>
<td>Urinary Tyrosine: Leucine ratio</td>
<td>3.63</td>
<td>2.22 &lt;0.02</td>
</tr>
<tr>
<td>% and ERPM Urinary Tyrosine</td>
<td>0.83%</td>
<td>0.53% &lt;0.03</td>
</tr>
<tr>
<td>% Neutrophils</td>
<td>57.3%</td>
<td>54.4% &lt;0.03</td>
</tr>
<tr>
<td>Serum Ph2a</td>
<td>11297</td>
<td>9222 &lt;0.03</td>
</tr>
<tr>
<td>Neutrophil count</td>
<td>3.56</td>
<td>3.17 &lt;0.05</td>
</tr>
<tr>
<td>Pesticide residue Deildrin</td>
<td>0.25</td>
<td>0.08 &lt;0.05</td>
</tr>
<tr>
<td>Decreased</td>
<td></td>
<td></td>
</tr>
<tr>
<td>% Urinary Phenylalanine</td>
<td>0.75%</td>
<td>0.91% &lt;0.03</td>
</tr>
<tr>
<td>% Urinary Proline</td>
<td>1.64%</td>
<td>2.02% &lt;0.03</td>
</tr>
<tr>
<td>% Urinary Asparagine</td>
<td>1.22%</td>
<td>1.64% &lt;0.04</td>
</tr>
<tr>
<td>% Lymphocytes</td>
<td>31.3%</td>
<td>33.8% &lt;0.04</td>
</tr>
<tr>
<td>Serum Phenylalanine</td>
<td>51809</td>
<td>61139 &lt;0.04</td>
</tr>
<tr>
<td>Serum Tyrosine: Leucine ratio</td>
<td>0.34</td>
<td>0.39 &lt;0.04</td>
</tr>
<tr>
<td>% Urinary Hydroxyproline</td>
<td>0.38%</td>
<td>0.51% &lt;0.05</td>
</tr>
<tr>
<td>% Urinary Alanine</td>
<td>5.28%</td>
<td>6.19% &lt;0.05</td>
</tr>
<tr>
<td><strong>Correlation Analysis</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Increased</td>
<td>r P</td>
<td>r P</td>
</tr>
<tr>
<td>Erythrocyte Sedimentation rate (ESR)</td>
<td>0.30 &lt;0.02</td>
<td>-</td>
</tr>
<tr>
<td>ALT</td>
<td>0.27 &lt;0.02</td>
<td>-</td>
</tr>
<tr>
<td>Potassium</td>
<td>0.26 &lt;0.02</td>
<td>-</td>
</tr>
<tr>
<td>Deildrin</td>
<td>0.25 &lt;0.02</td>
<td>-0.09 NS</td>
</tr>
<tr>
<td>Urine volume</td>
<td>0.24 &lt;0.03</td>
<td>0.06 NS</td>
</tr>
<tr>
<td>Basophil count</td>
<td>0.24 &lt;0.03</td>
<td>-0.10 NS</td>
</tr>
<tr>
<td>Neutrophil count</td>
<td>0.24 &lt;0.03</td>
<td>-0.17 NS</td>
</tr>
<tr>
<td>Serum S-Methylcysteine</td>
<td>0.24 &lt;0.03</td>
<td>-0.13 NS</td>
</tr>
<tr>
<td>% Urinary Acetaminophen</td>
<td>0.24 &lt;0.03</td>
<td>0.13 NS</td>
</tr>
<tr>
<td>% and ERPM Urinary Aspartic acid</td>
<td>0.24 &lt;0.03</td>
<td>-0.13 NS</td>
</tr>
<tr>
<td>ERPM Urinary Glutamic acid</td>
<td>0.22 &lt;0.05</td>
<td>-0.10 NS</td>
</tr>
<tr>
<td>DDE</td>
<td>0.22 &lt;0.05</td>
<td>0.14 NS</td>
</tr>
<tr>
<td>Decreased</td>
<td></td>
<td></td>
</tr>
<tr>
<td>% Lymphocytes</td>
<td>-0.22 &lt;0.04</td>
<td>-0.03 NS</td>
</tr>
<tr>
<td>Serum Proline</td>
<td>-0.22 &lt;0.05</td>
<td>-0.08 NS</td>
</tr>
<tr>
<td>% Urinary Hippuric acid</td>
<td>-0.22 &lt;0.04</td>
<td>0.17 NS</td>
</tr>
<tr>
<td>Lymphocyte count</td>
<td>-0.03 NS</td>
<td>-0.24 &lt;0.05</td>
</tr>
<tr>
<td>Leukocyte count</td>
<td>0.19 NS</td>
<td>-0.25 &lt;0.04</td>
</tr>
<tr>
<td>% Urinary Tyrosine</td>
<td>0.17 NS</td>
<td>-0.25 &lt;0.04</td>
</tr>
<tr>
<td>Serum Ph1</td>
<td>0.06 NS</td>
<td>-0.27 &lt;0.03</td>
</tr>
<tr>
<td>Serum β-Aminoisobutyrate</td>
<td>0.11 NS</td>
<td>-0.25 &lt;0.03</td>
</tr>
</tbody>
</table>

12-month facial pain frequency CFS patient regression model; R²=0.597, F=5.176, P<0.0002
Variables; 1) ESR P<0.009, 2) Excretion rate/min Aspartic acid P<0.0004, 3) Serum iron P<0.002.

12-month facial pain frequency Control subject regression model; R²=0.973, F=8.440, P<0.00008
Variables; 1) Serum Ph1 -P<0.003, 2) Leukocyte count -P<0.15, 3) Serum threonine P<0.0009.
3.4.2.m. 7-Day TMJ Pain Severity.

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

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

<table>
<thead>
<tr>
<th>Serum Parameter</th>
<th>TMJ Pain</th>
<th>No TMJ Pain</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>T-test 7-day TMJ pain positive v controls</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Increased</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serum Ph2a</td>
<td>12598(8542)</td>
<td>4433(3954) &lt;0.0007</td>
</tr>
<tr>
<td>Serum Ethanolamine</td>
<td>9033(4479)</td>
<td>5968(2709) &lt;0.008</td>
</tr>
<tr>
<td>% Urinary Tyrosine</td>
<td>0.99(1.03)</td>
<td>0.41(0.58) &lt;0.01</td>
</tr>
<tr>
<td>% Neutrophils</td>
<td>58.1(5.6)</td>
<td>52.4(8.3) &lt;0.02</td>
</tr>
<tr>
<td>Urinary tyrosine: leucine ratio</td>
<td>2.75(2.24)</td>
<td>1.51(2.50) &lt;0.02</td>
</tr>
<tr>
<td>ERPM Urinary Tyrosine</td>
<td>42.0(36.5)</td>
<td>22.9(38.1) &lt;0.02</td>
</tr>
<tr>
<td>ERPM Urinary Acetaminophen</td>
<td>26.7(75.9)</td>
<td>0.02(1.1) &lt;0.04</td>
</tr>
<tr>
<td>% Urinary UM15a</td>
<td>0.94(0.96)</td>
<td>0.44(0.30) &lt;0.04</td>
</tr>
<tr>
<td>Serum Ph1</td>
<td>35794(22203)</td>
<td>26597(11744) &lt;0.05</td>
</tr>
<tr>
<td>Decreased</td>
<td></td>
<td></td>
</tr>
<tr>
<td>% Urinary UM17</td>
<td>0.09(0.10)</td>
<td>0.15(0.10) &lt;0.02</td>
</tr>
<tr>
<td>Serum Phenylalanine</td>
<td>57357(30552)</td>
<td>65218(20369) &lt;0.03</td>
</tr>
<tr>
<td>Serum β-aminoisobutyrate</td>
<td>9606(4066)</td>
<td>12360(3720) &lt;0.03</td>
</tr>
<tr>
<td>Serum tyrosine: leucine ratio</td>
<td>0.37(0.18)</td>
<td>0.48(0.16) &lt;0.04</td>
</tr>
<tr>
<td><strong>Correlation Analysis</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Increased</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serum Ph1</td>
<td>0.36 &lt;0.02</td>
<td>0.09 NS</td>
</tr>
<tr>
<td>Serum Bilirubin</td>
<td>0.25 &lt;0.05</td>
<td>-</td>
</tr>
<tr>
<td>% and ERPM Urinary Acetaminophen</td>
<td>-0.02 NS</td>
<td>0.55 &lt;0.001</td>
</tr>
<tr>
<td>% Urinary β-aminoisobutyrate</td>
<td>-0.11 NS</td>
<td>0.37 &lt;0.009</td>
</tr>
<tr>
<td>% Urinary UM15a</td>
<td>0.15 NS</td>
<td>0.32 &lt;0.03</td>
</tr>
<tr>
<td>ERPM Urinary UM15</td>
<td>0.07 NS</td>
<td>0.28 &lt;0.03</td>
</tr>
</tbody>
</table>

**7-Day TMJ pain severity CFS patients** regression model; $R^2=0.612, F=4.575, P<0.0007$  Variables; 1) Excretion rate/min UM15 $P<0.22$, 2) bilirubin $P<0.0008$, 3) sodium -$P<0.0007$.

**7-Day TMJ pain severity Control subject** regression model; $R^2=0.928, F=13.619, P<0.00000$  Variables; 1) % urinary β-aminoisobutyrate $P<0.00002$, 2) serum proline $P<0.00001$, 3) Serum Ph2a $P<0.00003$.

The CFS TMJ pain group had an increase in the percentage neutrophils and the neutrophil: lymphocyte ratio as well as the urinary tyrosine: leucine ratio and a reduction in the serum tyrosine: leucine ratio. The serum levels of Ph1, Ph2a and ethanolamine were increased whilst the phenylalanine and β-aminoisobutyrate levels were reduced. They also had increases in the excretion rate per minute and relative abundance of tyrosine, as well as a reduction in the excretion rate per minute of acetaminophen and the relative abundance of UM17 and an increase in the relative abundance of UM15a.
The scalar 7-day TMJ pain response was assessed within the CFS patients and the control subjects. Table 3.19 indicates that forward stepwise multiple regression (all blood cell and biochemistry variables included) for the 7-day TMJ pain response was associated with differences in the other variables in both the CFS patients and the control subjects. In the CFS patients the primary parameter that determined the difference was an increase in the excretion rate per minute of UM15 followed by the bilirubin concentration. Univariate analysis (Spearman rank correlation) showed that the 7-day TMJ pain severity response was associated with increases in serum bilirubin and unknown Ph1. In the control subjects the primary variable for the forward stepwise regression analysis was the % urinary β-aminoisobutyrate. Univariate analysis was also applied to the 7-day TMJ pain severity response in the control subjects. The 7-day response was associated with an increase in UM15, the analgesic acetaminophen (paracetamol), β-aminoisobutyrate, UM15a, the percentage neutrophils and the neutrophil: lymphocyte ratio.

3.4.2.n. 12-Month TMJ Pain Severity.

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

3.4.2.o. 12-Month TMJ Pain Frequency.

The CFS patients who responded positively to the 12-month TMJ pain frequency scalar response were compared with the remaining CFS patients to assess which blood cell and biochemical parameters were associated with the frequency of reporting of TMJ pain.
Similarly the scalar responses to the 12-month frequency of TMJ pain were assessed by multivariate and univariate analyses in the CFS and control groups.

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

<table>
<thead>
<tr>
<th>Serum Parameter</th>
<th>CFS</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Severity Correlation Analysis</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Increased</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Erythrocyte Sedimentation rate (ESR)</td>
<td>0.30 &lt;0.01</td>
<td>-</td>
</tr>
<tr>
<td>Urine volume</td>
<td>0.27 &lt;0.02</td>
<td>0.04 NS</td>
</tr>
<tr>
<td>% and ERPM Urinary Acetaminophen</td>
<td>0.25 &lt;0.03</td>
<td>-0.04 NS</td>
</tr>
<tr>
<td>Serum S-methylcysteine</td>
<td>0.24 &lt;0.03</td>
<td>-0.09 NS</td>
</tr>
<tr>
<td>AST</td>
<td>0.23 &lt;0.04</td>
<td>-</td>
</tr>
<tr>
<td>Protein</td>
<td>0.22 &lt;0.04</td>
<td>-</td>
</tr>
<tr>
<td>% Urinary Aconitic acid</td>
<td>-0.07 NS</td>
<td>0.23 &lt;0.05</td>
</tr>
<tr>
<td><strong>Decreased</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serum Ph1</td>
<td>0.17 NS</td>
<td>-0.23 &lt;0.05</td>
</tr>
</tbody>
</table>

**12-month TMJ pain severity CFS patient** regression model; R²=0.528, F=3.910, P<0.002
Variables: 1) ESR P<0.03, 2) AST P<0.003, 3) Serum β-aminoisobutyrate P<0.004.

**12-month TMJ pain severity Control subject** regression model; R²=0.985, F=23.020, P<0.00000
Variables: 1) serum Ph1 P<0.04, 2) % urine Aconitic acid P<0.04, 3) Serum aspartic acid P<0.03.

<table>
<thead>
<tr>
<th>Frequency T-Test Analysis</th>
<th>TMJ Pain</th>
<th>No TMJ Pain</th>
</tr>
</thead>
<tbody>
<tr>
<td>Increased</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serum Ph2a</td>
<td>11873</td>
<td>8375 &lt;0.003</td>
</tr>
<tr>
<td>Serum S-Methylcysteine</td>
<td>1477</td>
<td>623 &lt;0.006</td>
</tr>
<tr>
<td>MCV</td>
<td>88.2</td>
<td>86.7 &lt;0.009</td>
</tr>
<tr>
<td>% and ERPM Urinary Acetaminophen</td>
<td>2.00%</td>
<td>0.29% &lt;0.02</td>
</tr>
<tr>
<td>% and ERPM Urinary 3-Methylhistidine</td>
<td>2.47%</td>
<td>1.89% &lt;0.02</td>
</tr>
<tr>
<td>% Urinary Ethanolamine</td>
<td>3.40%</td>
<td>2.58% &lt;0.04</td>
</tr>
<tr>
<td>MCH</td>
<td>30.2</td>
<td>29.7 &lt;0.04</td>
</tr>
<tr>
<td>Heptochlor</td>
<td>0.20</td>
<td>0.05 &lt;0.04</td>
</tr>
<tr>
<td>Decreased</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serum Tyrosine: Leucine ratio</td>
<td>0.33</td>
<td>0.41 &lt;0.002</td>
</tr>
<tr>
<td>Serum Tyrosine</td>
<td>26244</td>
<td>33581 &lt;0.008</td>
</tr>
</tbody>
</table>

**Correlation Analysis**

<table>
<thead>
<tr>
<th></th>
<th>CFS</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Increased</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basophil count</td>
<td>0.27 &lt;0.02</td>
<td>-0.07 NS</td>
</tr>
<tr>
<td>Mean corpuscular volume (MCV)</td>
<td>0.26 &lt;0.02</td>
<td>0.05 NS</td>
</tr>
<tr>
<td>Erythrocyte Sedimentation rate (ESR)</td>
<td>0.25 &lt;0.05</td>
<td>-</td>
</tr>
<tr>
<td>Eosinophil count</td>
<td>0.23 &lt;0.04</td>
<td>-0.07 NS</td>
</tr>
<tr>
<td>Serum Glutamic acid</td>
<td>0.23 &lt;0.04</td>
<td>0.06 NS</td>
</tr>
<tr>
<td>Serum S-methylcysteine</td>
<td>0.26 &lt;0.02</td>
<td>-0.12 NS</td>
</tr>
<tr>
<td>Serum Lysine</td>
<td>0.26 &lt;0.02</td>
<td>0.08 NS</td>
</tr>
<tr>
<td><strong>Decreased</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glycine</td>
<td>-0.29 &lt;0.008</td>
<td>-0.11 NS</td>
</tr>
<tr>
<td>Ph1</td>
<td>0 NS</td>
<td>-0.25 &lt;0.03</td>
</tr>
</tbody>
</table>

**12-month TMJ pain frequency CFS patient** regression model; R²=0.535, F=4.031, P<0.001
Variables: 1) Serum glycine -P<0.25, 2) ESR P<0.01, 3) Serum s-methylcysteine P<0.02.

**12-month TMJ pain frequency Control subject** regression model; R²=0.999, F=195.11, P<0.00001
Variables: 1) Serum Ph1 P<0.00002, 2) Monocyte count -P<0.000009, 3) Serum tryptophan -P<0.00005.
Table 3.20 shows that forward stepwise multiple regression analyses of the CFS patients (all blood cell and serum biochemistry and the urinary relative abundance variables included) and control subjects have different parameter profiles in association with the 12-month TMJ response. The primary parameter that determined the difference was a reduction in serum glycine, followed by the ESR. Univariate analysis (Spearman rank correlation) showed that the 12-month frequency of TMJ pain was associated with increases in the ESR, the basophil and eosinophil counts, mean red cell volume, serum s-methylcysteine, lysine and the excitatory amino acid, glutamic acid. A reduction in serum glycine levels was also noted. In the control subjects a reduction in serum unknown Ph1 was the principle factor associated with the increase in 12-month TMJ pain frequency.

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

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

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

<table>
<thead>
<tr>
<th>SCL-90-R Question</th>
<th>7-Day Severity</th>
<th>12-month Frequency</th>
<th>12-month Severity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Q40 Nausea or upset stomach</td>
<td>r: -0.31, P &lt; 0.004</td>
<td>r: -0.29, P &lt; 0.008</td>
<td>r: -0.23, P &lt; 0.04</td>
</tr>
<tr>
<td>Q17 Trembling</td>
<td>r: -0.34, P &lt; 0.001</td>
<td>r: -0.25, P &lt; 0.03</td>
<td>-</td>
</tr>
<tr>
<td>Q111 Hair loss</td>
<td>r: -0.35, P &lt; 0.001</td>
<td>r: -0.27, P &lt; 0.002</td>
<td>-</td>
</tr>
<tr>
<td>Q36 Feeling others are unsympathetic or do not understand</td>
<td>-</td>
<td>r: -0.29, P &lt; 0.008</td>
<td>r: -0.28, P &lt; 0.01</td>
</tr>
<tr>
<td>Q114 Leg cramps</td>
<td>-</td>
<td>r: -0.24, P &lt; 0.03</td>
<td>r: -0.22, P &lt; 0.04</td>
</tr>
<tr>
<td>Q19 Poor appetite</td>
<td>r: -0.30, P &lt; 0.005</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Q115 Muscle twitches</td>
<td>r: -0.27, P &lt; 0.03</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Q123 Facial muscle pain or discomfort</td>
<td>r: -0.26, P &lt; 0.05</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Q52 Numbness or tingling in parts of your body</td>
<td>r: -0.24, P &lt; 0.03</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Q4 Faintness or dizziness</td>
<td>r: -0.22, P &lt; 0.05</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Q75 Feeling nervous when left alone</td>
<td>r: -0.21, P &lt; 0.05</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Q23 Suddenly scared for no reason</td>
<td>-</td>
<td>r: -0.22, P &lt; 0.05</td>
<td>-</td>
</tr>
<tr>
<td>Q124 Swallowing difficulties</td>
<td>-</td>
<td>r: -0.22, P &lt; 0.05</td>
<td>-</td>
</tr>
<tr>
<td>Q9 Trouble remembering things</td>
<td>-</td>
<td>r: -0.21, P &lt; 0.05</td>
<td>-</td>
</tr>
<tr>
<td>Q103 Sore glands</td>
<td>-</td>
<td>r: -0.21, P &lt; 0.05</td>
<td>-</td>
</tr>
<tr>
<td>Q42 Soreness of your muscles</td>
<td>-</td>
<td>-</td>
<td>r: -0.25, P &lt; 0.03</td>
</tr>
<tr>
<td>Q113 Joint pain</td>
<td>-</td>
<td>-</td>
<td>r: -0.22, P &lt; 0.04</td>
</tr>
</tbody>
</table>

7-day Severity regression model; R²=0.953, F=26.436, P<0.00000
Variables; 1) Mind going blank -P<1.0E-12, 2) Facial pain -P<1.7E-10, 3) Heart racing/pounding P<0.000002
12-Month Frequency regression model; R²=0.812, F=12.493, P<0.00000
Variables; 1) Hair loss -P<0.000001, 2) Swallowing difficulties -P<0.000004, 3) Q35 P<0.00002
12-Month Severity regression model; R²=0.934, F=41.707, P<0.00000
Variables; 1) Hair loss -P<0.000001, 2) Others unsympathetic -P<0.000006, 3) Q24 P<0.05
The fall in serum sodium levels was associated with increases in the 7-day severity of dysregulated appetite, nausea, muscle symptoms (including facial pain), faintness and dizziness and paraesthesia. The fall in serum sodium levels was also associated with increases in the 12-month frequency and severity of nausea, musculoskeletal symptoms, hair loss and cognitive symptoms. These symptom associations with sodium are very similar to the symptoms associated with facial pain as shown in Tables 2.3 and 2.9 (Chapter 2; pages 26 and 36, respectively). These symptom data support the association between falling serum sodium levels and facial muscle pain.

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

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

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Facial Pain</th>
<th>No Facial Pain</th>
<th>Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Increased</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>U % &amp; EPM Succinic acid</td>
<td>0.75 &lt;0.002</td>
<td>-0.17 NS</td>
<td>&lt;0.002</td>
</tr>
<tr>
<td>U % &amp; EPM β-alanine</td>
<td>0.71 &lt;0.005</td>
<td>-0.30 &lt;0.04</td>
<td>&lt;0.0008</td>
</tr>
<tr>
<td>U % &amp; EPM UM15</td>
<td>0.67 &lt;0.009</td>
<td>-0.28 NS</td>
<td>&lt;0.002</td>
</tr>
<tr>
<td>U % &amp; EPM Glycine</td>
<td>0.66 &lt;0.01</td>
<td>0.20 NS</td>
<td>NS</td>
</tr>
<tr>
<td>U EPM Leucine</td>
<td>0.65 &lt;0.02</td>
<td>-0.26 NS</td>
<td>&lt;0.003</td>
</tr>
<tr>
<td>U % &amp; EPM Alanine</td>
<td>0.63 &lt;0.02</td>
<td>-0.11 NS</td>
<td>&lt;0.02</td>
</tr>
<tr>
<td>U EPM Valine</td>
<td>0.60 &lt;0.03</td>
<td>-0.19 NS</td>
<td>&lt;0.02</td>
</tr>
<tr>
<td>U EPM Phenylacetic acid</td>
<td>0.59 &lt;0.03</td>
<td>-0.23 NS</td>
<td>&lt;0.009</td>
</tr>
<tr>
<td>Creatinine</td>
<td>0.57 &lt;0.04</td>
<td>0.16 NS</td>
<td>NS</td>
</tr>
<tr>
<td>Mean Platelet volume</td>
<td>0.55 &lt;0.04</td>
<td>0.03 NS</td>
<td>NS</td>
</tr>
<tr>
<td>U EPM S-Methylcysteine</td>
<td>0.52 NS</td>
<td>-0.20 NS</td>
<td>&lt;0.03</td>
</tr>
<tr>
<td>Amino acids / minute</td>
<td>0.50 NS</td>
<td>-0.16 NS</td>
<td>&lt;0.04</td>
</tr>
<tr>
<td>Chloride</td>
<td>0.50 NS</td>
<td>0.29 &lt;0.05</td>
<td>NS</td>
</tr>
<tr>
<td>ALT</td>
<td>0.48 NS</td>
<td>0.01 NS</td>
<td>NS</td>
</tr>
<tr>
<td>Iron</td>
<td>0.46 NS</td>
<td>-0.27 NS</td>
<td>&lt;0.03</td>
</tr>
<tr>
<td>mls / minute</td>
<td>0.37 NS</td>
<td>-0.35 &lt;0.02</td>
<td>&lt;0.03</td>
</tr>
<tr>
<td>U % &amp; EPM UM15a</td>
<td>0.35 NS</td>
<td>-0.36 &lt;0.02</td>
<td>&lt;0.04</td>
</tr>
<tr>
<td>Bicarbonate</td>
<td>0.09 NS</td>
<td>0.06 NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

Decreased

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Facial Pain</th>
<th>No Facial Pain</th>
<th>Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Organic acids / minute</td>
<td>0 NS</td>
<td>-0.29 &lt;0.05</td>
<td>NS</td>
</tr>
<tr>
<td>U % &amp; EPM Hippuric acid</td>
<td>-0.11 NS</td>
<td>-0.30 &lt;0.04</td>
<td>NS</td>
</tr>
<tr>
<td>ESR</td>
<td>-0.55 NS</td>
<td>0.24 NS</td>
<td>&lt;0.02</td>
</tr>
<tr>
<td>Platelet number</td>
<td>-0.56 &lt;0.04</td>
<td>0.10 NS</td>
<td>&lt;0.04</td>
</tr>
<tr>
<td>Serum Lysine</td>
<td>-0.61 &lt;0.02</td>
<td>-0.04 NS</td>
<td>NS</td>
</tr>
<tr>
<td>U%, EPM 1-Methylhistidine</td>
<td>-0.36 NS</td>
<td>-0.27 NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

Facial Pain regression model: R²=0.973, F=14.163, P<0.07
Variables: 1) % U glycine P<0.03, 2) Serum S-methylcysteine P<0.06, 3) % U UM15 P<0.02

No Facial Pain regression model: R²=0.941, F=6.433, P<0.02
Variables: 1) urine volume -P<0.12, 2) iron P<0.05, 3) % U Hippuric acid P<0.002

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

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

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

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

Table 3.24 shows that the increase in neutrophil levels in the facial pain positive subjects was associated with increased vitamin B12 levels, Ph2a, leukocyte and monocyte count and reductions in serum phosphate and the urinary relative abundance of threonine and hydroxyproline. In the CFS patients without a 7-day facial pain response increases in the neutrophil count correlated with 15 different measures, whilst differences in correlations were found for 9 different measures.
Eleven metabolites were statistically different between the facial pain patients and those CFS patients without facial pain. Large differences were found for 2 urinary metabolites (UM28 and threonine) and serum vitamin B12. Thus the increase in the neutrophil count in the facial pain patients was associated with large differences in associations with many different metabolites and immune parameters indicating that the neutrophil count in the facial pain patients was associated with a dysregulation of metabolism. These data confirm the findings that increases in the 12-month frequency and severity of facial pain are associated with increases in the neutrophil count and that these factors are associated with alterations in vitamin B12, serum phosphate and alterations in connective tissue amino acids proline and hydroxyproline.

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

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

7-day Severity regression model; $R^2=0.705$, $F(10,44)=10.512$, $P<0.00000$
Variables; 1) Spells of terror or panic $P<0.01$, 2) Stomach cramps $P=0.02$, 3) Blurry vision $P<0.006$

12-Month Frequency regression model; $R^2=0.938$, $F=21.348$, $P<0.00000$
Variables; 1) Uncomfortable about eating $P<0.000001$, 2) Blaming yourself $P<0.000001$, 3) Diarrhoea $P<0.000001$

12-Month Severity regression model; $R^2=0.900$, $F=12.165$, $P<0.00000$
Variables; 1) Uncomfortable about eating $P<0.05$, 2) Sore glands $P<0.000003$, 3) Thoughts not your own $P<0.48$
Table 3.24. Summary of the differences in correlation coefficients for the neutrophil count between CFS patients with a positive 7-day facial pain response compared with those with no response.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Facial Pain</th>
<th>No Facial pain</th>
<th>Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>r</td>
<td>P</td>
<td>r</td>
</tr>
<tr>
<td><strong>Increased</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leukocyte count</td>
<td>0.84</td>
<td>&lt;0.001</td>
<td>0.88</td>
</tr>
<tr>
<td>Vitamin B12</td>
<td>0.78</td>
<td>&lt;0.008</td>
<td>0.12</td>
</tr>
<tr>
<td>Serum Ph2a</td>
<td>0.61</td>
<td>&lt;0.02</td>
<td>0.01</td>
</tr>
<tr>
<td>Monocyte count</td>
<td>0.58</td>
<td>&lt;0.03</td>
<td>0.53</td>
</tr>
<tr>
<td>% U UM28</td>
<td>0.51</td>
<td>NS</td>
<td>-0.35</td>
</tr>
<tr>
<td>% U Hippuric acid</td>
<td>0.42</td>
<td>NS</td>
<td>-0.37</td>
</tr>
<tr>
<td>Basophil count</td>
<td>0.38</td>
<td>NS</td>
<td>0.44</td>
</tr>
<tr>
<td>Eosinophil count</td>
<td>0.22</td>
<td>NS</td>
<td>0.29</td>
</tr>
<tr>
<td>Serum Ph2</td>
<td>0.21</td>
<td>NS</td>
<td>-0.32</td>
</tr>
<tr>
<td>Platelet count</td>
<td>0.05</td>
<td>NS</td>
<td>0.49</td>
</tr>
<tr>
<td><strong>Decreased</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>% U Lysine</td>
<td>-0.15</td>
<td>NS</td>
<td>0.31</td>
</tr>
<tr>
<td>% U Glycine</td>
<td>-0.24</td>
<td>NS</td>
<td>0.30</td>
</tr>
<tr>
<td>% U UM14</td>
<td>-0.27</td>
<td>NS</td>
<td>0.30</td>
</tr>
<tr>
<td>Serum Phenylalanine</td>
<td>-0.31</td>
<td>NS</td>
<td>0.30</td>
</tr>
<tr>
<td>% U UM13a</td>
<td>-0.38</td>
<td>NS</td>
<td>0.30</td>
</tr>
<tr>
<td>U % &amp; EPM Proline</td>
<td>-0.46</td>
<td>NS</td>
<td>0.19</td>
</tr>
<tr>
<td>U % &amp; EPM Threonine</td>
<td>-0.61</td>
<td>&lt;0.03</td>
<td>0.35</td>
</tr>
<tr>
<td>% U Hydroxyproline</td>
<td>-0.63</td>
<td>&lt;0.02</td>
<td>0.06</td>
</tr>
<tr>
<td>Serum Phosphate</td>
<td>-0.61</td>
<td>&lt;0.02</td>
<td>0.02</td>
</tr>
<tr>
<td>Serum β-aminoisobutyrate</td>
<td>0.28</td>
<td>NS</td>
<td>-0.34</td>
</tr>
</tbody>
</table>

**Facial Pain** regression model: $R^2=0.999, F=44693, P<0.00000$
Variables: 1) Leukocyte count $P<0.000002$, 2) % lymphocytes $P<0.000004$, 3) ESR $P<0.000006$

**No Facial Pain** regression model: $R^2=0.999, F=8468.2, P<0.00000$
Variables: 1) Leukocyte count $P<0.000001$, 2) % lymphocytes $P<0.000001$, 3) eosinophil count $P<0.000002$

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

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

Table 3.25 shows that increases in the basophil count in the CFS patients were associated with increases in the 7-day symptom severity of bladder infections and sleep disturbance and the 12-month frequency/ severity of facial muscle and TMJ pain, muscle soreness, diarrhoea and hair loss. The previously reported pilot study association between facial pain and bladder infections/cystitis was noted (McGregor et al, 1992). Thus increases in the basophil count are highly associated with TMD symptoms and a segment of those symptoms reported by TMD patients.
Table 3.25. Summary of the correlation analyses of the association between the SCL-90-R scalar symptom responses and the basophil count.

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

7-day Severity regression model; $R^2=0.791$, $F(12,42)=13.262$, $P<0.00000$
Variables; 1) Hair loss $P<0.03$, 2) Frightening thoughts or images $P<0.04$, 3) Heartburn/gastric reflux $P<0.04$

12-Month Frequency regression model; $R^2=0.953$, $F=28.489$, $P<0.00000$
Variables; 1) Bladder infections/cystitis $P<0.000001$, 2) Feeling fearful $P<0.75$, 3) Thoughts of death or dying $P<0.00002$

12-Month Severity regression model; $R^2=0.976$, $F=40.973$, $P<0.00000$
Variables; 1) Facial pain $P<0.0006$, 2) Recurrent genital infections $P<0.78$, 3) Stomach cramps $P<0.02$

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

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Facial Pain</th>
<th>No Facial Pain</th>
<th>Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>r</td>
<td>P</td>
<td></td>
</tr>
<tr>
<td><strong>Increased</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Deildrin</td>
<td>0.72</td>
<td>&lt;0.003</td>
<td></td>
</tr>
<tr>
<td>Urine volume</td>
<td>0.57</td>
<td>&lt;0.04</td>
<td></td>
</tr>
<tr>
<td>Total serum amino acids</td>
<td>0.54</td>
<td>&lt;0.05</td>
<td></td>
</tr>
<tr>
<td>Serum S-methylcysteine</td>
<td>0.53</td>
<td>&lt;0.05</td>
<td></td>
</tr>
<tr>
<td>Leukocyte count</td>
<td>0.38</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Neutrophil count</td>
<td>0.38</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>% Urinary Acetaminophen</td>
<td>0.33</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Monocyte count</td>
<td>0.32</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>% Urinary CFSUM1</td>
<td>0.17</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Eosinophil count</td>
<td>0.05</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Lymphocyte count</td>
<td>0.03</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Thyroid stimulating hormone</td>
<td>0.02</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Platelet count</td>
<td>-0.06</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Urinary EPM UM13a</td>
<td>-0.11</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Urinary EPM S-methylcysteine</td>
<td>-0.11</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Urinary EPM proline</td>
<td>-0.15</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Urinary EPM glutamic acid</td>
<td>-0.28</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Urinary EPM phenylalanine</td>
<td>-0.30</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Urinary EPM UM15a</td>
<td>-0.37</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>MCV</td>
<td>-0.43</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Urinary metabolite/minute</td>
<td>-0.50</td>
<td>NS</td>
<td>&lt;0.03</td>
</tr>
<tr>
<td>Urinary EPM tyrosine</td>
<td>-0.51</td>
<td>NS</td>
<td>&lt;0.03</td>
</tr>
<tr>
<td>Urinary organic acids/minute</td>
<td>-0.52</td>
<td>NS</td>
<td>&lt;0.04</td>
</tr>
<tr>
<td>Urinary EPM aspartic acid</td>
<td>-0.52</td>
<td>NS</td>
<td>&lt;0.02</td>
</tr>
<tr>
<td><strong>Decreased</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Red cell count</td>
<td>-0.22</td>
<td>NS</td>
<td>&lt;0.03</td>
</tr>
<tr>
<td>Urinary EPM Aconitic acid</td>
<td>-0.56</td>
<td>&lt;0.04</td>
<td>&lt;0.02</td>
</tr>
<tr>
<td>% and EPM Urinary Citric acid</td>
<td>-0.74</td>
<td>&lt;0.003</td>
<td>&lt;0.006</td>
</tr>
</tbody>
</table>

**Facial Pain** regression model; $R^2 = 0.525$, $F=6.637$, $P<0.05$
Variables; 1) Deildrin $P<0.04$

**No Facial Pain** regression model; $R^2 = 0.924$, $F=4.835$, $P<0.03$
Variables; 1) Leukocyte count $P<0.003$, 2) % U acetaminophen $P<0.006$, 3) TSH $P<0.02$

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

An increase in ALT was positively associated with the 12-month severity and frequency of facial pain (Table 3.17), which may be the result of a blunted nitric oxide response (Kayashima et al, 1995; Mena et al, 1996; Bruunsgaard et al, 1997). Table 3.25 shows the correlation analyses for serum ALT levels and the 7-day and 12-month severity and frequency symptom responses for the CFS patients. Table 3.27 shows that increasing ALT levels were associated with alterations in TMD, musculoskeletal, sleep disturbance and depression and mood alteration symptoms, including most associated with depression. The questions normally associated with depression, feelings of worthlessness, guilt and the lack of motivation were associated with this tissue damage marker (ALT). This shows that the RDC/TMD Axis II depression assessment is associated with biochemical changes consistent with tissue or liver damage offering a potential biochemical basis for depression.
Table 3.27. Summary of the correlation analyses of the association between the SCL-90-R scalar symptom responses and ALT.

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

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

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

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Facial Pain</th>
<th>No Facial pain</th>
<th>Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Increased</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AST</td>
<td>0.78 &lt;0.001</td>
<td>0.63 &lt;0.001</td>
<td>NS</td>
</tr>
<tr>
<td>Urea</td>
<td>0.73 &lt;0.003</td>
<td>0.31 &lt;0.04</td>
<td>NS</td>
</tr>
<tr>
<td>S-Methylcysteine</td>
<td>0.60 &lt;0.03</td>
<td>-0.03 NS</td>
<td>&lt;0.04</td>
</tr>
<tr>
<td>% Neutrophils</td>
<td>0.58 &lt;0.03</td>
<td>0.16 NS</td>
<td>NS</td>
</tr>
<tr>
<td>% &amp; EPM U Glutamic acid</td>
<td>0.57 &lt;0.04</td>
<td>0.38 &lt;0.007</td>
<td>NS</td>
</tr>
<tr>
<td>Creatinine</td>
<td>0.43 NS</td>
<td>0.39 &lt;0.006</td>
<td>NS</td>
</tr>
<tr>
<td>GGT</td>
<td>0.39 NS</td>
<td>0.60 &lt;0.001</td>
<td>NS</td>
</tr>
<tr>
<td>mls Urine/minute</td>
<td>0.28 NS</td>
<td>0.37 &lt;0.008</td>
<td>NS</td>
</tr>
<tr>
<td>% U Aspartic acid</td>
<td>0.27 NS</td>
<td>0.38 &lt;0.008</td>
<td>NS</td>
</tr>
<tr>
<td>Serum ethanolamine</td>
<td>0.22 NS</td>
<td>0.29 &lt;0.05</td>
<td>NS</td>
</tr>
<tr>
<td>Haematocrit</td>
<td>0.16 NS</td>
<td>0.44 &lt;0.002</td>
<td>NS</td>
</tr>
<tr>
<td>Haemoglobin</td>
<td>0.15 NS</td>
<td>0.47 &lt;0.001</td>
<td>NS</td>
</tr>
<tr>
<td>DDE</td>
<td>0.05 NS</td>
<td>0.38 &lt;0.009</td>
<td>NS</td>
</tr>
<tr>
<td>Red cell count</td>
<td>0.03 NS</td>
<td>0.38 &lt;0.006</td>
<td>NS</td>
</tr>
<tr>
<td>% U Lysine</td>
<td>-0.13 NS</td>
<td>0.31 &lt;0.04</td>
<td>NS</td>
</tr>
<tr>
<td><strong>Decreased</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>U EPM Hippuric acid</td>
<td>-0.06 NS</td>
<td>-0.30 &lt;0.04</td>
<td>NS</td>
</tr>
<tr>
<td>Chloride</td>
<td>0.33 NS</td>
<td>-0.31 &lt;0.03</td>
<td>NS</td>
</tr>
<tr>
<td>Eosinophil count</td>
<td>0.01 NS</td>
<td>-0.31 &lt;0.04</td>
<td>NS</td>
</tr>
<tr>
<td>Serum Serine</td>
<td>-0.23 NS</td>
<td>-0.32 &lt;0.03</td>
<td>NS</td>
</tr>
<tr>
<td>% U Phenyllamine</td>
<td>-0.29 NS</td>
<td>0.30 &lt;0.04</td>
<td>NS</td>
</tr>
<tr>
<td>Serum Phosphate</td>
<td>-0.42 NS</td>
<td>-0.39 &lt;0.008</td>
<td>NS</td>
</tr>
<tr>
<td>Serum Glutamic acid</td>
<td>-0.51 NS</td>
<td>0.13 NS</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Serum Lysine</td>
<td>-0.54 &lt;0.05</td>
<td>0.13 NS</td>
<td>&lt;0.04</td>
</tr>
<tr>
<td>Platelet count</td>
<td>-0.55 &lt;0.05</td>
<td>0.05 NS</td>
<td>NS</td>
</tr>
<tr>
<td>Serum Tyrosine: leucine</td>
<td>-0.56 &lt;0.04</td>
<td>0.08 NS</td>
<td>&lt;0.04</td>
</tr>
<tr>
<td>Lymphocyte count</td>
<td>-0.58 &lt;0.04</td>
<td>-0.18 NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

**Facial Pain** regression model; $R^2=0.946, F=23.248, P<0.006$
Variables; 1) AST $P<0.003$, 2) Serum Tryptophan $P<0.01$, 3) serum lysine -$P<0.03$

**No Facial Pain** regression model; $R^2=0.979, F=34.415, P<0.0000$
Variables; 1) AST $P<0.000004$, 2) GGT $P<0.00005$, 3) Serum serine -$P<0.0006$

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

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

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

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

Aspartic acid
7-day Severity regression model; R²=0.869, F(16,38)=15.815, P<0.00000
Variables; 1) Nightmares P<0.000001, 2) Sore throat P<0.0001, 3) Constipation P<0.000001
12-Month Frequency regression model; R²=0.972, F=43.956, P<0.00000
Variables; 1) Feelings of worthlessness P<0.0000001, 2) Poor circulation P<0.000001, 3) Joint pain P<0.000003
12-Month Severity regression model; R²=0.956, F=27.026, P<0.00000
Variables; 1) Uneasy in crowds P<0.00000003, 2) Headaches P<0.14, 3) Paranoia -P<0.000001

Table 3.30 shows that the increases in the excretion rate per minute of both aspartic acid and glutamine/glutamic acid are highly correlated with the excretion of many metabolite as well as the total urinary metabolites and total urinary amino acids and therefore reflect the increased excretion of metabolite. Twelve biochemical parameters had different correlation coefficients between the facial pain and no facial pain CFS patients. These include the urine volume, vitamin B12, DDE, alanine, asparagine, aconitic acid and several leukocyte parameters (basophils, monocytes and lymphocytes). The changes in metabolites associated with increases in the excretion rate per minute of aspartic acid were predominantly associated with the increase in metabolites associated with increasing urine volume. This is very similar to that noted in the RDC/TMD type Ia group analysis.
Table 3.30. Summary of the differences in correlation coefficients for aspartic acid between CFS patients with a positive 7-day facial pain response compared with those with no response.

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

**Facial Pain** regression model:
Variables: 1) $P<0.000001$, 2) $P<0.000001$, 3) $P<0.000001$

**No Facial Pain** regression model:
Variables: 1) $P<0.000001$, 2) $P<0.000004$, 3) $P<0.00002$
3.4.2.u. Association between Serum Lysine, Symptoms and Biochemical Parameters.

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

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

<table>
<thead>
<tr>
<th>SCL-90-R Question</th>
<th>7-Day Severity</th>
<th>12-month Frequency</th>
<th>12-month Severity</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Increased</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Q105 Jaw joint pain or tenderness</td>
<td>- r P</td>
<td>0.29 &lt;0.007</td>
<td>-</td>
</tr>
<tr>
<td>Q40 Nausea or upset stomach</td>
<td>-</td>
<td>0.24 &lt;0.02</td>
<td>-</td>
</tr>
<tr>
<td><strong>Decreased</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Q65 Having to repeat the same actions such as touching</td>
<td>-0.25 &lt;0.02</td>
<td>-0.27 &lt;0.01</td>
<td>-0.26 &lt;0.02</td>
</tr>
<tr>
<td>Q69 Feeling very self-conscious with others</td>
<td>-0.25 &lt;0.02</td>
<td>-0.21 &lt;0.05</td>
<td>-</td>
</tr>
<tr>
<td>Q21 Feeling shy or uneasy with the opposite sex</td>
<td>-0.23 &lt;0.03</td>
<td>-0.25 &lt;0.02</td>
<td>-</td>
</tr>
<tr>
<td>Q70 Feeling uneasy in crowds, such as shopping</td>
<td>-0.23 &lt;0.03</td>
<td>-0.26 &lt;0.02</td>
<td>-</td>
</tr>
<tr>
<td>Q59 Thoughts of death or dying</td>
<td>-</td>
<td>-0.28 &lt;0.01</td>
<td>-0.25 &lt;0.02</td>
</tr>
<tr>
<td>Q112 Heartburn / Gastric reflux</td>
<td>-</td>
<td>-0.24 &lt;0.03</td>
<td>-0.22 &lt;0.04</td>
</tr>
<tr>
<td>Q124 Swallowing difficulties</td>
<td>-0.40 &lt;0.001</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Q41 Feeling inferior to others</td>
<td>-0.28 &lt;0.007</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Q106 Fatigue</td>
<td>-0.30 &lt;0.02</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Q73 Feeling uncomfortable about eating or drinking in public</td>
<td>-0.25 &lt;0.02</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Q100 Confusion</td>
<td>-0.26 &lt;0.04</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Q103 Sore glands</td>
<td>-0.26 &lt;0.04</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Q92 Allergies</td>
<td>-0.25 &lt;0.04</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Q10 Worried about sloppiness or carelessness</td>
<td>-0.21 &lt;0.04</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Q115 Muscle twitches</td>
<td>-0.24 &lt;0.05</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Q116 Nightmares</td>
<td>-0.25 &lt;0.05</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Q4 Faintness or dizziness</td>
<td>-</td>
<td>-0.29 &lt;0.007</td>
<td>-</td>
</tr>
<tr>
<td>Q2 Nervousness or shakiness inside</td>
<td>-</td>
<td>-0.25 &lt;0.02</td>
<td>-</td>
</tr>
<tr>
<td>Q25 Feeling afraid to go out of your house alone</td>
<td>-</td>
<td>-0.26 &lt;0.02</td>
<td>-</td>
</tr>
<tr>
<td>Q28 Feeling blocked in getting things done</td>
<td>-</td>
<td>-0.24 &lt;0.03</td>
<td>-</td>
</tr>
<tr>
<td>Q47 Feeling afraid to travel on buses, subways or trains</td>
<td>-</td>
<td>-0.23 &lt;0.03</td>
<td>-</td>
</tr>
<tr>
<td>Q45 Having to check and double-check what you do</td>
<td>-</td>
<td>-</td>
<td>-0.29 &lt;0.007</td>
</tr>
<tr>
<td>Q38 Having to do things very slowly to ensure correctness</td>
<td>-</td>
<td>-</td>
<td>-0.26 &lt;0.02</td>
</tr>
<tr>
<td>Q55 Trouble concentrating</td>
<td>-</td>
<td>-</td>
<td>-0.21 &lt;0.05</td>
</tr>
</tbody>
</table>

7-day Severity regression model; R²=0.914, F=13.762, P<0.000000
Variables; 1) Nightmares -P<0.0000004, 2) Bladder infections/cystitis P<0.000002, 3) Frightening thoughts or images P<0.000005

12-Month Frequency regression model; R²=0.964, F=37.378, P<0.000000
Variables; 1) Heartburn/gastric reflux -P<0.0000001, 2) Nausea P<0.0000001, 3) Faintness/dizziness -P<0.000001

12-Month Severity regression model; R²=0.930, F=17.833, P<0.000000
Variables; 1) Someone else controlling thoughts -P<0.0000001, 2) Abdominal pain P<0.0000001, 3) Heartburn/gastric reflux -P<0.00001

Table 3.31 shows that the increase in serum lysine is associated with TMJ pain and nausea. However, falls in serum lysine levels were associated with fatigue, psychological, cognitive and musculoskeletal symptoms. Table 3.31 shows that within the facial pain
patients serum lysine was positive associated with serum ornithine, serum aconitic acid and serum tryptophan and negatively associated with sodium, serum glycine, vitamin B12 and ALT. Correlation coefficient differences between the facial pain and no facial pain patients were identified for 9 parameters. These included sodium, ALT, vitamin B12, platelet count and volume, serum tyrosine, creatinine, urea and the urinary excretion rate per minute of β-alanine.

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

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

Facial Pain regression model; R²=0.998, F=152.39, P<0.0001
Variables; 1) Serum Glutamic acid P<0.00007, 2) Serum Ornithine P<0.00002, 3) CFSUM1 -P<0.03
No Facial Pain regression model; R²=0.979, F=53.171, P<0.00000
Variables; 1) Serum Tryptophan P<0.000001, 2) Serum asparagine -P<0.00003, 3) % U Phenylacetic acid P<0.000001.

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

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

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

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

Table 3.34 summarises the biochemical parameter associations with increasing urine volume in the CFS patients with a positive 7-day face pain response and the remaining CFS patients. In the patients with face pain the primary multiple regression variable for increasing
Table 3.33. Summary of the correlation analyses of the association between the SCL-90-R scalar symptom responses and urine volume.

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

7-day Severity regression model: R²=0.931, F=17.472, *P*<0.00000
Variables: 1) Weak in parts of body *P*<0.000002, 2) Someone controls your thoughts *P*<0.000003, 3) Frequent arguments *P*<0.43

12-Month Frequency regression model: R²=0.943, F=21.514, *P*<0.00000
Variables: 1) Rashes *P*<0.009, 2) Uneasy about people watching or talking about you *P*<0.000001, 3) Spells of terror or panic *P*<0.000001

12-Month Severity regression model: R²=0.878, F=8.984, *P*<0.00000
Variables: 1) Nightmares *P*<0.0002, 2) Easily annoyed or irritated *P*<0.93, 3) Hopeless about future *P*<0.72

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

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

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

Facial Pain regression model; R²=0.956, F=16.456, P<0.03
Variables; 1) Serum protein -P<0.006, 2) Serum leucine -P<0.02, 3) Serum tryptophan -P<0.03
No Facial Pain regression model; R²=0.791, F=6.162, P<0.003
Variables; 1) %U lysine P<0.002, 2) Serum proline -P<0.006, 3) TSH P<0.004

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

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

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

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

**7-day Severity** regression model; R²=0.722, F(12,42)=9.103, P<0.000000
Variables; 1) Constipation -P<0.000001, 2) Heartburn/gastric reflux P<0.000005, 3) Poor circulation P<0.00000001

**12-Month Frequency** regression model; R²=0.963, F=36,133, P<0.000000
Variables; 1) Trouble getting breath P<0.0000001, 2) Suddenly scared for no reason -P<0.0000001, 3) Frightening thoughts or images P<0.0000001

**12-Month Severity** regression model; R²=0.959, F=31,058, P<0.000000
Variables; 1) Others unsympathetic -P<0.02, 2) Thoughts of death or dying P<0.0000002, 3) Feeling lonely even when with people P<0.04
In the CFS patients multiple regression analysis identified a negative correlation with constipation as the primary symptom associated with DDE levels in the 7-day symptom severity analysis. In the 12-month frequency and severity multiple regression analysis, “trouble getting your breath” and “others being unsympathetic”, respectively, were the primary regression questionnaire symptoms associated with DDE.

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

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

<table>
<thead>
<tr>
<th>Parameter</th>
<th>CFS</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>R²=0.535, F=2.832, P&lt;0.009</td>
<td></td>
</tr>
<tr>
<td>Positive Correlations</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Variables</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1) Serum Proline -P&lt;0.01, 2) Serum S-methylcysteine P&lt;0.04, 3) Glucose P&lt;0.07</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Variables</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1) Urine volume -P&lt;0.0002, 2) %U Threonine -P&lt;0.0004, 3) % Monocytes P&lt;0.000001</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Variables</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1) Serum Proline -P&lt;0.01, 2) Serum S-methylcysteine P&lt;0.04, 3) Glucose P&lt;0.07</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Variables</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1) Urine volume -P&lt;0.0002, 2) %U Threonine -P&lt;0.0004, 3) % Monocytes P&lt;0.000001</td>
<td></td>
</tr>
</tbody>
</table>

NA = Not available
DDE correlated with age in all study subjects and with illness duration in the CFS patients. In the CFS group the primary multiple regression parameter was a reduction in serum proline whilst in the control group the primary discriminant variable was urine volume. In the uni-variant analysis DDE was positively associated with glucose, ALT, % basophils, the urinary tyrosine: leucine ratio, serum ethanolamine, s-methylcysteine and Ph2a, and the urinary relative abundance of acetaminophen and glutamic acid. DDE was negative correlated with serum proline and serine, and the urinary relative abundance of UM13 and UM14 as well as the urinary excretion rate per minute of CFSUM1 and UM28.

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

<table>
<thead>
<tr>
<th>SCL-90-R Question</th>
<th>7-Day Severity</th>
<th>12-month Frequency</th>
<th>12-month Severity</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>CFS Patients</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Q86 Thoughts or images of a frightening nature</td>
<td>0.27 &lt;0.02</td>
<td>0.27 &lt;0.01</td>
<td>0.22 &lt;0.04</td>
</tr>
<tr>
<td>Q60 Overeating</td>
<td>0.25 &lt;0.02</td>
<td>0.26 &lt;0.02</td>
<td>0.23 &lt;0.03</td>
</tr>
<tr>
<td>Q63 Having urges to beat injury or harm somebody</td>
<td>-</td>
<td>0.24 &lt;0.03</td>
<td>0.22 &lt;0.04</td>
</tr>
<tr>
<td>Q122 Earaches</td>
<td>0.26 &lt;0.05</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Q26 Blaming yourself for things</td>
<td>0.26 &lt;0.02</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Q80 Feeling that something bad is going to happen to you</td>
<td>0.25 &lt;0.02</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Q5 Loss of sexual interest or pleasure</td>
<td>0.24 &lt;0.03</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Q33 Feeling fearful</td>
<td>0.22 &lt;0.04</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Q6 Feeling critical of others</td>
<td>0.21 &lt;0.05</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Q64 Awakening early in the morning</td>
<td>-</td>
<td>0.25 &lt;0.02</td>
<td>-</td>
</tr>
<tr>
<td>Q89 Feelings of guilt</td>
<td>-</td>
<td>0.24 &lt;0.03</td>
<td>-</td>
</tr>
<tr>
<td>Q123 Facial pain</td>
<td>-</td>
<td>0.23 &lt;0.04</td>
<td>-</td>
</tr>
<tr>
<td>Q67 Having urges to smash or break things</td>
<td>-</td>
<td>-</td>
<td>0.26 &lt;0.01</td>
</tr>
<tr>
<td>Q10 Worried about sloppiness or carelessness</td>
<td>-</td>
<td>-</td>
<td>0.22 &lt;0.05</td>
</tr>
<tr>
<td>Q24 Temper outbursts that you cannot control</td>
<td>-</td>
<td>-</td>
<td>0.22 &lt;0.05</td>
</tr>
<tr>
<td><strong>Control Subjects</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Q103 Sore glands</td>
<td>0.31 &lt;0.008</td>
<td>0.33 &lt;0.005</td>
<td>-</td>
</tr>
<tr>
<td>Q93 Constipation</td>
<td>0.29 &lt;0.02</td>
<td>0.29 &lt;0.02</td>
<td>-</td>
</tr>
<tr>
<td>Q124 Swallowing difficulties</td>
<td>0.28 &lt;0.02</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Q53 A lump in your throat</td>
<td>0.26 &lt;0.03</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Q57 Feeling tense or keyed up</td>
<td>0.24 &lt;0.04</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Q13 Feeling afraid of open spaces</td>
<td>-</td>
<td>0.40 &lt;0.0005</td>
<td>-</td>
</tr>
</tbody>
</table>

**7-day Severity** regression model; \( R^2=0.955, F(16,38)=50.212, P<0.000001 \)
Variables: 1) Frightening thoughts or images \( P<0.000001 \), 2) Trouble falling asleep \( P<0.000001 \), 3) Idea being punished for sins \( P<0.000001 \)

**12-Month Frequency** regression model; \( R^2=0.947, F=24.980, P<0.000000 \)
Variables: 1) Constipation \( P<0.000002 \), 2) Difficulty making decisions \( P<0.000001 \), 3) Avoiding certain things or places because they frighten you \( P<0.000001 \)

**12-Month Severity** regression model; \( R^2=0.934, F=18.527, P<0.000000 \)
Variables: 1) Feeling low in energy \( P<0.000002 \), 2) Lump in throat \( P<0.02 \), 3) Thoughts of ending your life \( P<0.002 \)

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

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

<table>
<thead>
<tr>
<th>Parameter</th>
<th>CFS</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Age</td>
<td>0.23 &lt;0.03</td>
</tr>
<tr>
<td></td>
<td>Duration</td>
<td>0.01</td>
</tr>
<tr>
<td><strong>Positive Correlations</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serum S-Methylcysteine</td>
<td>0.60 &lt;0.001</td>
<td>0.15</td>
</tr>
<tr>
<td>% Urine Ornithine</td>
<td>0.36 &lt;0.001</td>
<td>0.15</td>
</tr>
<tr>
<td>Total serum organic acids</td>
<td>0.34 &lt;0.001</td>
<td>0.05</td>
</tr>
<tr>
<td>% Urine Ethanolamine</td>
<td>0.34 &lt;0.001</td>
<td>-0.01</td>
</tr>
<tr>
<td>% Urine Acetaminophen</td>
<td>0.27 &lt;0.007</td>
<td>-0.06</td>
</tr>
<tr>
<td>% Urine UM15a</td>
<td>0.26 &lt;0.02</td>
<td>-0.08</td>
</tr>
<tr>
<td>Serum Aspartic acid</td>
<td>0.24 &lt;0.02</td>
<td>-0.13</td>
</tr>
<tr>
<td>% Urine Tyrosine</td>
<td>0.23 &lt;0.03</td>
<td>-0.01</td>
</tr>
<tr>
<td>% Urine UM15</td>
<td>0.22 &lt;0.04</td>
<td>0.01</td>
</tr>
<tr>
<td>Serum Ph2a</td>
<td>0.02</td>
<td>0.26 &lt;0.02</td>
</tr>
<tr>
<td>MCHC</td>
<td>0.11</td>
<td>0.25 &lt;0.03</td>
</tr>
<tr>
<td>% Urine Aconitic acid</td>
<td>-0.01</td>
<td>0.23 &lt;0.05</td>
</tr>
<tr>
<td><strong>Negative Correlations</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>% urine UM14</td>
<td>-0.26 &lt;0.02</td>
<td>0.02</td>
</tr>
<tr>
<td>EPM urine UM28</td>
<td>-0.25 &lt;0.02</td>
<td>0.09</td>
</tr>
<tr>
<td>SerumAlanine</td>
<td>-0.24 &lt;0.02</td>
<td>-0.11</td>
</tr>
</tbody>
</table>

**CFS regression model:** $R^2=0.870$, $F=4.476$, $P<0.0009$
**Variables:** 1) Serum S-methylcysteine $P<0.0005$, 2) %U Ornithine $P<0.002$, 3) %U 1-methylhistidine $P<0.004$

**Control regression model:** $R^2=0.608$, $F=3.225$, $P<0.0002$
**Variables:** 1) Serum Ph2a $P<0.000005$, 2) % U lysine $P<0.05$, 3) % U Tyrosine $P<0.008$

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

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

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

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

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Antibiotic Use</th>
<th>No Antibiotics</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Multiple Antibiotics</td>
<td>DDE Mean (Std.Err.)</td>
<td>Mean (Std.Err.)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Increased</td>
<td>4.46(1.04)</td>
<td>2.48(0.41)</td>
<td></td>
</tr>
<tr>
<td>Total Urinary organic acids excreted/minute</td>
<td>3871(525)</td>
<td>2772(441)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Urinary EPM UM15a</td>
<td>33.1(9.8)</td>
<td>18.9(3.4)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Decreased</td>
<td>Serum aspartic acid</td>
<td>1372(128)</td>
<td>1912(277)</td>
</tr>
<tr>
<td></td>
<td>Serum serine</td>
<td>1469(131)</td>
<td>1769(184)</td>
</tr>
<tr>
<td></td>
<td>% U proline</td>
<td>1.61(0.13)</td>
<td>2.17(0.23)</td>
</tr>
<tr>
<td></td>
<td>%U β-alanine</td>
<td>0.81(0.09)</td>
<td>1.14(0.14)</td>
</tr>
<tr>
<td>Prolonged Antibiotics (&gt;4 weeks)</td>
<td>Increased</td>
<td>DDE Mean (Std.Err.)</td>
<td>Mean (Std.Err.)</td>
</tr>
<tr>
<td></td>
<td>% Basophils</td>
<td>0.40(0.04)</td>
<td>0.26(0.04)</td>
</tr>
<tr>
<td></td>
<td>% Lymphocytes</td>
<td>34.1(1.15)</td>
<td>30.4(1.36)</td>
</tr>
<tr>
<td></td>
<td>Serum Tyrosine: leucine</td>
<td>0.35()</td>
<td>0.29()</td>
</tr>
<tr>
<td>Decreased</td>
<td>Serum Isoleucine</td>
<td>1540(86.8)</td>
<td>1426(96.1)</td>
</tr>
<tr>
<td></td>
<td>Neutrophil: lymphocyte ratio</td>
<td>1.74(0.10)</td>
<td>2.15(0.15)</td>
</tr>
</tbody>
</table>

Multiple Antibiotics Forward stepwise regression model: R²=0.754, F=3.825, P<0.001
Serum aspartic acid -P<0.003; 2) Serum serine -P<0.0005; 3) Serum leucine -P<0.05

Prolonged Antibiotics Forward stepwise regression model: R²=0.721, F=3.224, P<0.004
DDE P<0.49; 2) Serum valine -P<0.00004; 3) Serum PH2 P<0.0003

<table>
<thead>
<tr>
<th>Prolonged Stress</th>
<th>Stress</th>
<th>No Stress</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Increased</td>
<td>Bilirubin</td>
<td>11.9(1.32)</td>
<td>8.8(0.49)</td>
</tr>
<tr>
<td></td>
<td>Serum glycine</td>
<td>3741(247)</td>
<td>3363(257)</td>
</tr>
<tr>
<td>Decreased</td>
<td>Serum protein</td>
<td>71.7(0.65)</td>
<td>73.5(0.59)</td>
</tr>
</tbody>
</table>

Prolonged Stress Forward stepwise regression model: R²=0.716, F=3.149, P<0.004
1) Bilirubin P<0.00005; 2) Serum protein -P<0.03; 3) ALT P<0.09
Multiple regression analysis revealed that the fall in serum aspartic acid levels was the primary variable associated with multiple antibiotic use whilst an increase in DDE was the primary variable associated with prolonged antibiotic use. CFS patients who reported multiple antibiotic use had increases in DDE, urinary relative abundance of UM15a and the total urinary organic acids excreted and reductions in serum aspartic acid and serum and urinary relative abundance of proline and \(\beta\)-alanine. CFS patients taking prolonged courses of antibiotics had increases in DDE, percentage basophils and lymphocytes and an increase in the serum tyrosine: leucine ratio, as well as reductions in serum isoleucine and the neutrophil: lymphocyte ratio. Thus prolonged use of antibiotics was associated with increases in the percentage basophils and DDE and a reduction in the neutrophil: lymphocyte ratio.

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

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

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

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

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

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Somatization dimension</th>
<th>Depression dimension</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Correlation</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Increased</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urine volume</td>
<td>0.32 &lt;0.002</td>
<td></td>
</tr>
<tr>
<td>ESR</td>
<td>0.24 &lt;0.05</td>
<td></td>
</tr>
<tr>
<td>Total leukocyte count</td>
<td>0.22 &lt;0.04</td>
<td></td>
</tr>
<tr>
<td>Serum Ph1</td>
<td>0.21 &lt;0.04</td>
<td></td>
</tr>
<tr>
<td><strong>Decreased</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serum Sodium</td>
<td>-0.23 &lt;0.03</td>
<td></td>
</tr>
<tr>
<td>Serum Alanine</td>
<td>-0.23 &lt;0.03</td>
<td></td>
</tr>
<tr>
<td>Mean erythrocyte haemoglobin</td>
<td>-0.22 &lt;0.03</td>
<td></td>
</tr>
<tr>
<td><strong>Somatization</strong></td>
<td>Forward stepwise regression model: $R^2=0.741, F=3.572, P&lt;0.002$</td>
<td></td>
</tr>
<tr>
<td>1) Urine volume $P&lt;0.05$</td>
<td>2) ESR $P&lt;0.0001$</td>
<td></td>
</tr>
<tr>
<td>3) Serum Proline $P&lt;0.01$</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Correlation</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Increased</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serum potassium</td>
<td>0.24 &lt;0.03</td>
<td></td>
</tr>
<tr>
<td>Mean Platelet Volume</td>
<td>0.23 &lt;0.02</td>
<td></td>
</tr>
<tr>
<td>Eosinophil count</td>
<td>0.23 &lt;0.03</td>
<td></td>
</tr>
<tr>
<td><strong>Decreased</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>% urinary Proline</td>
<td>-0.31 &lt;0.003</td>
<td></td>
</tr>
<tr>
<td>% urinary hydroxyproline</td>
<td>-0.29 &lt;0.006</td>
<td></td>
</tr>
<tr>
<td>MCHC</td>
<td>-0.29 &lt;0.01</td>
<td></td>
</tr>
<tr>
<td>Pesticide Heptochlor</td>
<td>-0.22 &lt;0.04</td>
<td></td>
</tr>
<tr>
<td>% urinary threonine</td>
<td>-0.21 &lt;0.05</td>
<td></td>
</tr>
<tr>
<td>Pesticide Aldrin</td>
<td>-0.21 &lt;0.05</td>
<td></td>
</tr>
<tr>
<td><strong>Depression</strong></td>
<td>Forward stepwise regression model: $R^2=0.580, F=2.768, P&lt;0.009$</td>
<td></td>
</tr>
<tr>
<td>1) % urinary Proline $P&lt;0.001$</td>
<td>2) MCHC $P&lt;0.23$</td>
<td></td>
</tr>
<tr>
<td>3) Eosinophil count $P&lt;0.03$</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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

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

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

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Female Model/Variables</th>
<th>Male Model/Variables</th>
</tr>
</thead>
<tbody>
<tr>
<td>Face Pain</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7-day Severity</td>
<td>$R^2=0.994, F=176.92, P&lt;0.000000$ Variables</td>
<td>$R^2=0.770, F=8.918, P&lt;0.0007$ Variables</td>
</tr>
<tr>
<td></td>
<td>1) Sodium, $-&lt;1.0E^{-13}$</td>
<td>1) Serum Lysine $-&lt;0.004$</td>
</tr>
<tr>
<td></td>
<td>2) Creatinine, $+&lt;6.0E^{-10}$</td>
<td>2) Serum aminohydroxyproprionate $-&lt;0.04$</td>
</tr>
<tr>
<td></td>
<td>3) Calcium, $+&lt;2.0E^{-10}$</td>
<td>3) Neutrophil count $+&lt;0.05$</td>
</tr>
<tr>
<td>12-month Severity</td>
<td>$R^2=0.988, F=54.067, P&lt;0.000000$ Variables</td>
<td>$R^2=0.862, F=18.691, P&lt;0.00004$ Variables</td>
</tr>
<tr>
<td></td>
<td>1) ALT $+&lt;0.002$</td>
<td>1) Haemoglobin $+&lt;0.00001$</td>
</tr>
<tr>
<td></td>
<td>2) Protein $+&lt;0.0003$</td>
<td>2) Red cell distribution width $+&lt;0.002$</td>
</tr>
<tr>
<td></td>
<td>3) Iron $+&lt;0.000002$</td>
<td>3) ANA Titre $-&lt;0.0007$</td>
</tr>
<tr>
<td>12-month Frequency</td>
<td>$R^2=0.981, F=24.931, P&lt;0.00001$ Variables</td>
<td>$R^2=0.758, F=13.548, P&lt;0.00003$ Variables</td>
</tr>
<tr>
<td></td>
<td>1) Protein $+&lt;0.05$</td>
<td>1) Haemoglobin $+&lt;0.0002$</td>
</tr>
<tr>
<td></td>
<td>2) Basophil count $+&lt;0.33$</td>
<td>2) Red cell distribution width $+&lt;0.002$</td>
</tr>
<tr>
<td></td>
<td>3) Serum Proline $-&lt;0.001$</td>
<td>3) Haematocrit $-&lt;0.003$</td>
</tr>
<tr>
<td>TMJ Pain</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7-day Severity</td>
<td>$R^2=0.964, F=38.893, P&lt;0.000000$ Variables</td>
<td>$R^2=0.681, F=21.305, P&lt;0.001$ Variables</td>
</tr>
<tr>
<td></td>
<td>1) Serum alanine $+&lt;0.0000008$</td>
<td>1) Deildrin $+&lt;0.001$</td>
</tr>
<tr>
<td></td>
<td>2) Serum Vit B12 $-&lt;0.0000003$</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3) Serum serine $+&lt;0.00000004$</td>
<td></td>
</tr>
<tr>
<td>12-month Severity</td>
<td>$R^2=0.998, F=357.73, P&lt;0.000000$ Variables</td>
<td>$R^2=0.953, F=44.072, P&lt;0.000000$ Variables</td>
</tr>
<tr>
<td></td>
<td>1) Serum glycine $-&lt;1.5E^{-11}$</td>
<td>1) Excret. Rate/minute glycine $+&lt;1.0E^{-6}$</td>
</tr>
<tr>
<td></td>
<td>2) Serum β-aminobutyrate $+&lt;1.7E^{-12}$</td>
<td>2) Vit B12 $+&lt;0.0003$</td>
</tr>
<tr>
<td></td>
<td>3) ESR $+&lt;1.0E^{-14}$</td>
<td>3) Serum alanine $-&lt;0.000007$</td>
</tr>
<tr>
<td>12-month Frequency</td>
<td>$R^2=0.985, F=14.161, P&lt;0.004$ Variables</td>
<td>$R^2=0.916, F=32.925, P&lt;0.000000$ Variables</td>
</tr>
<tr>
<td></td>
<td>1) Protein $+&lt;0.002$</td>
<td>1) Iron $-&lt;0.0000005$</td>
</tr>
<tr>
<td></td>
<td>2) Acetaminophen $+&lt;0.19$</td>
<td>2) Haematocrit $+&lt;0.0002$</td>
</tr>
<tr>
<td></td>
<td>Serum Ph1 $+&lt;0.22$</td>
<td>3) Bilirubin $+&lt;0.0003$</td>
</tr>
</tbody>
</table>

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

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

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

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

The cellular and biochemical variables associated with TMD symptoms in females appear to be distinct from the cellular and biochemical variables in males. These data also show variations in the white blood cell parameters and urinary metabolite output between males and females.
Table 3.42. Analysis of the differences in Spearman rank correlation analyses for the various TMD symptoms between females and males in the CFS patient group.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Female 7-day Severity</th>
<th>Female 12-month Severity</th>
<th>Female 12-month Frequency</th>
<th>Male 7-day Severity</th>
<th>Male 12-month Severity</th>
<th>Male 12-month Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Facial Pain</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>-</td>
<td>0.27 &lt; 0.03</td>
<td>-</td>
<td>-</td>
<td>0.26 NS</td>
<td>-</td>
</tr>
<tr>
<td>Duration</td>
<td>-</td>
<td>0.30 &lt; 0.02</td>
<td>0.25 &lt; 0.05</td>
<td>-</td>
<td>0.30 NS</td>
<td>0.29 NS</td>
</tr>
<tr>
<td>Symptom prevalence</td>
<td>-</td>
<td>0.30 &lt; 0.02</td>
<td>0.30 &lt; 0.02</td>
<td>-</td>
<td>0.78 &lt; 0.00002</td>
<td>0.71 &lt; 0.0003</td>
</tr>
<tr>
<td>White cell count</td>
<td>-</td>
<td>0.12 NS</td>
<td>-</td>
<td>-</td>
<td>0.46 &lt; 0.03</td>
<td>-</td>
</tr>
<tr>
<td>Neutrophil:Lymphocyte</td>
<td>-</td>
<td>-</td>
<td>0.09 NS</td>
<td>-</td>
<td>-</td>
<td>0.53 &lt; 0.01</td>
</tr>
<tr>
<td>Neutrophil count</td>
<td>-</td>
<td>0.12 NS</td>
<td>0.14 NS</td>
<td>-</td>
<td>0.52 &lt; 0.01</td>
<td>0.48 &lt; 0.03</td>
</tr>
<tr>
<td>Lymphocyte count</td>
<td>-</td>
<td>-</td>
<td>-0.12 NS</td>
<td>-</td>
<td>-</td>
<td>-0.50 &lt; 0.02</td>
</tr>
<tr>
<td>Basophil count</td>
<td>-</td>
<td>0.31 &lt; 0.01</td>
<td>0.26 &lt; 0.04</td>
<td>-</td>
<td>0.45 &lt; 0.04</td>
<td>0.48 &lt; 0.03</td>
</tr>
<tr>
<td>Sodium</td>
<td>-0.36 &lt; 0.02</td>
<td>-</td>
<td>-</td>
<td>0.11 NS</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>ALT</td>
<td>-</td>
<td>0.36 &lt; 0.005</td>
<td>0.27 &lt; 0.04</td>
<td>-</td>
<td>0.55 &lt; 0.009</td>
<td>0.40 NS</td>
</tr>
<tr>
<td>AST</td>
<td>-</td>
<td>0.34 &lt; 0.007</td>
<td>-</td>
<td>-</td>
<td>0.30 NS</td>
<td>-</td>
</tr>
<tr>
<td>Iron</td>
<td>-</td>
<td>0.31 &lt; 0.02</td>
<td>0.30 &lt; 0.02</td>
<td>-</td>
<td>-0.35 NS</td>
<td>-0.23 NS</td>
</tr>
<tr>
<td>Urea</td>
<td>-</td>
<td>0.30 &lt; 0.02</td>
<td>-</td>
<td>-</td>
<td>0.09 NS</td>
<td>-</td>
</tr>
<tr>
<td>Protein</td>
<td>-</td>
<td>0.26 &lt; 0.05</td>
<td>0.28 &lt; 0.03</td>
<td>-</td>
<td>-0.04 NS</td>
<td>-0.34 NS</td>
</tr>
<tr>
<td>Potassium</td>
<td>-</td>
<td>-</td>
<td>0.20 NS</td>
<td>-</td>
<td>-</td>
<td>0.44 &lt; 0.05</td>
</tr>
<tr>
<td>Glucose</td>
<td>-</td>
<td>-</td>
<td>0.09 NS</td>
<td>-</td>
<td>-</td>
<td>0.44 &lt; 0.05</td>
</tr>
<tr>
<td>Urine volume</td>
<td>-</td>
<td>0.32 &lt; 0.01</td>
<td>0.30 &lt; 0.02</td>
<td>-</td>
<td>0.06 NS</td>
<td>0.10 NS</td>
</tr>
</tbody>
</table>

| TMJ Pain             |                       |                          |                           |                     |                        |                         |
| Symptom prevalence   | -                     | 0.12 NS                  | 0.33 < 0.008              | 0.32 < 0.009        | -                      | 0.39 NS                | 0.36 NS                 |
| Neutrophil:Lymphocyte| -0.26 NS              | 0.09 NS                  | -                         | 0.48 < 0.05         | -                      | -                      | -                       |
| Monocyte count       | -0.15 NS              | -                        | 0.05 NS                   | 0.49 < 0.05         | -                      | -0.48 < 0.03           | -                       |
| Red cell distribution width | -0.15 NS | - | 0.09 NS | - | 0.47 < 0.03 | - | - | - |
| Haematocrit          | -                     | 0.24 NS                  | 0.30 < 0.02               | 0.49 < 0.05         | -                      | -0.48 < 0.03           | -                       |
| Deildrin             | -0.19 NS              | 0.08 NS                  | 0.30 < 0.02               | -                   | -0.45 < 0.05           | -                      | -                       |
| Iron                 | 0.33 < 0.03           | -                        | 0.05 NS                   | -                   | -                      | -                      | -                       |
| Metabolite/minute    | -                     | 0.04 NS                  | 0.06 NS                   | -                   | 0.44 < 0.05            | 0.63 < 0.003           | -                       |
| Amino acids/minute   | -                     | -                        | 0 NS                      | -                   | -                      | 0.56 < 0.02            | -                       |
| Organic acids/minute | -                     | -                        | -0.01 NS                  | -                   | -                      | 0.50 < 0.03            | -                       |
However, increases in the basophil count and the tissue damage marker, ALT, were prominent features of 12-month facial pain severity and frequency in both males and females. The reduction in sodium levels was a predominant feature of TMD expression in females whilst increases in neutrophil numbers with TMD were more prominent in males.

3.4.3. RNase-L Study.

3.4.3.a. Group Differences.

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

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

<table>
<thead>
<tr>
<th>Parameter</th>
<th>CFS</th>
<th>Control</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient Characteristics</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number</td>
<td>31</td>
<td>31</td>
<td>NS</td>
</tr>
<tr>
<td>Age</td>
<td>46.1±13(X±SD)</td>
<td>40.0±13(X±SD)</td>
<td>NS</td>
</tr>
<tr>
<td>% Female</td>
<td>71.9%</td>
<td>83.9%</td>
<td>NS</td>
</tr>
<tr>
<td>Prevalence</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Increased RNase-L activity</td>
<td>23 (71.9%)</td>
<td>4 (12.9%)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>sIL-2r (&gt;2SD)</td>
<td>13 (40.6%)</td>
<td>2 (6.5%)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Presence of IL-6</td>
<td>18 (56.3%)</td>
<td>3 (9.7%)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Concentration</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>sIL-2r</td>
<td>1465±999</td>
<td>711±292</td>
<td>&lt;0.0002</td>
</tr>
<tr>
<td>IL-6</td>
<td>70.94±108.33</td>
<td>8.58±28.52</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>TMD symptoms</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7-Day face pain</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prevalence</td>
<td>14 (43.8%)</td>
<td>2 (9.7%)</td>
<td>&lt;0.003</td>
</tr>
<tr>
<td>Severity score</td>
<td>0.88 (1.2)</td>
<td>0.16 (0.6)</td>
<td>&lt;0.003</td>
</tr>
<tr>
<td>TMJ lock/click</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prevalence</td>
<td>10 (31.3%)</td>
<td>6 (19.4%)</td>
<td>NS</td>
</tr>
<tr>
<td>Severity score</td>
<td>0.59 (1.1)</td>
<td>0.39 (1.0)</td>
<td>NS</td>
</tr>
<tr>
<td>Correlation Analysis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7-Day Face pain</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RNase-L</td>
<td>-0.10 NS</td>
<td>-0.03 NS</td>
<td>0.14 NS</td>
</tr>
<tr>
<td>sIL-2r</td>
<td>0.10 NS</td>
<td>-0.02 NS</td>
<td>0.24 NS</td>
</tr>
<tr>
<td>IL-6</td>
<td>-0.10 NS</td>
<td>-0.11 NS</td>
<td>0.10 NS</td>
</tr>
<tr>
<td>7-Day TMJ locking/clicking</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RNase-L</td>
<td>0.07 NS</td>
<td>-0.03 NS</td>
<td>0.09 NS</td>
</tr>
<tr>
<td>sIL-2r</td>
<td>-0.07 NS</td>
<td>-0.23 NS</td>
<td>-0.04 NS</td>
</tr>
<tr>
<td>IL-6</td>
<td>0.05 NS</td>
<td>-0.16 NS</td>
<td>0.05 NS</td>
</tr>
</tbody>
</table>

Group Forward stepwise discriminant function analysis; Wilks’ λ =0.643, F(3,58)=10.736, P<0.0000

Variables 1) RNase-L P<0.03; 2) sIL-2r P<0.004; 3) IL-6 P<0.02

Face pain Forward stepwise regression analysis; R²=0.046, F=2.876, P<0.10 (Model not significant)

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

### 3.4.3.b. TMD Symptom Associations.

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

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

<table>
<thead>
<tr>
<th>Assessment</th>
<th>All Subjects</th>
<th>CFS</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(r)</td>
<td>(P)</td>
<td>(r)</td>
</tr>
<tr>
<td>RNase-L</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Headaches</td>
<td>0.38</td>
<td>&lt;0.002</td>
<td>-0.01</td>
</tr>
<tr>
<td>Neck pain</td>
<td>0.27</td>
<td>&lt;0.04</td>
<td>0.14</td>
</tr>
<tr>
<td>Shoulder pain</td>
<td>0.28</td>
<td>&lt;0.03</td>
<td>0.12</td>
</tr>
<tr>
<td>Arm pain</td>
<td>0.44</td>
<td>&lt;0.0004</td>
<td>0.17</td>
</tr>
<tr>
<td>Abdominal pain</td>
<td>0.26</td>
<td>&lt;0.04</td>
<td>0.11</td>
</tr>
<tr>
<td>Leg pain</td>
<td>0.44</td>
<td>&lt;0.0003</td>
<td>0.22</td>
</tr>
<tr>
<td>Myalgia</td>
<td>0.42</td>
<td>&lt;0.0006</td>
<td>0.11</td>
</tr>
<tr>
<td>Muscle weakness</td>
<td>0.46</td>
<td>&lt;0.0002</td>
<td>0.19</td>
</tr>
<tr>
<td>Paraesthesia</td>
<td>0.49</td>
<td>&lt;0.00005</td>
<td>0.43</td>
</tr>
<tr>
<td>Fever</td>
<td>0.38</td>
<td>&lt;0.002</td>
<td>0.18</td>
</tr>
</tbody>
</table>
Table 3.44b. Summary of the Spearman correlation analysis associations between symptoms and sIL-2r and IL-6 in all subjects, the CFS patients and control subjects.

<table>
<thead>
<tr>
<th>Assessment</th>
<th>All Subjects</th>
<th>CFS</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>r</td>
<td>P</td>
<td>r</td>
</tr>
<tr>
<td>Soluble IL-2 receptor</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Headaches</td>
<td>0.34</td>
<td>&lt;0.007</td>
<td>0.01</td>
</tr>
<tr>
<td>Neck pain</td>
<td>0.42</td>
<td>&lt;0.0007</td>
<td>0.28</td>
</tr>
<tr>
<td>Shoulder pain</td>
<td>0.43</td>
<td>&lt;0.0004</td>
<td>0.40</td>
</tr>
<tr>
<td>Abdominal pain</td>
<td>0.32</td>
<td>&lt;0.02</td>
<td>0.34</td>
</tr>
<tr>
<td>Low back pain</td>
<td>0.45</td>
<td>&lt;0.0003</td>
<td>0.19</td>
</tr>
<tr>
<td>Leg pain</td>
<td>0.43</td>
<td>&lt;0.0006</td>
<td>0.36</td>
</tr>
<tr>
<td>Sciatica</td>
<td>0.53</td>
<td>&lt;0.0001</td>
<td>0.53</td>
</tr>
<tr>
<td>Arthritis</td>
<td>0.49</td>
<td>&lt;0.0006</td>
<td>0.40</td>
</tr>
<tr>
<td>Myalgia</td>
<td>0.44</td>
<td>&lt;0.0004</td>
<td>0.19</td>
</tr>
<tr>
<td>Muscle weakness</td>
<td>0.40</td>
<td>&lt;0.002</td>
<td>0.14</td>
</tr>
<tr>
<td>Paraesthesia</td>
<td>0.29</td>
<td>&lt;0.03</td>
<td>0.22</td>
</tr>
<tr>
<td>Fever</td>
<td>0.35</td>
<td>&lt;0.006</td>
<td>0.23</td>
</tr>
<tr>
<td>IL-6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arm pain</td>
<td>0.31</td>
<td>&lt;0.02</td>
<td>0.08</td>
</tr>
<tr>
<td>Low back pain</td>
<td>0.25</td>
<td>&lt;0.05</td>
<td>0.02</td>
</tr>
<tr>
<td>Myalgia</td>
<td>0.36</td>
<td>&lt;0.004</td>
<td>-0.07</td>
</tr>
<tr>
<td>Muscle weakness</td>
<td>0.33</td>
<td>&lt;0.009</td>
<td>-0.08</td>
</tr>
<tr>
<td>Paraesthesia</td>
<td>0.29</td>
<td>&lt;0.03</td>
<td>0.13</td>
</tr>
</tbody>
</table>