Pain Intensity, Illness Duration, and Protein Catabolism in Temporomandibular Disorder Patients with Chronic Muscle Pain

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Aims: *To investigate whether the duration of chronic pain in temporomandibular disorder (TMD) patients is associated with a net depletion of amino acids, and a distinct process from pain intensity.* ***Methods:*** *Twenty-nine patients defined by the research diagnostic criteria/TMD as having Type 1a muscle pain (TMD1A group), and 34 age- and sex-matched control subjects, were assessed for variation in urinary organic and amino acid excretion by gas chromatography-mass spectrometry.* ***Results:*** *The TMD1A patients' mean pain intensity, assessed on a visual analog scale (VAS), was 5.4 (95% confidence limits: 4.5 to 6.3), TMD1A illness duration was 5.0 ± 1.2 (SD) years, number of body areas with pain/subject was 6.3 ± 2.4 (range 0 to 10), and symptom prevalence from the Symptom Check List-90-Revised (SCL-90-R) was 25.5 ± 11.3 symptoms/subject, which was higher than the controls (5.2 ± 5.0 symptoms/subject, $P < .001$). TMD1A patient illness duration was positively correlated with symptom prevalence and body pain distribution, and all were independent of pain intensity. The TMD1A patients had: (1) an increased tyrosine:leucine ratio; and (2) reduced leucine concentrations (both $P < .001$), which suggests deregulated catabolism. Pain intensity was associated with: (1) changes in the multivariate urinary metabolite excretion patterns ($P < .001$); (2) reduced leucine concentrations ($P < .001$); and (3) increases in total urinary metabolites ($P < .04$), and in 2 unidentified molecules, UM28 ($P < .001$) and CFSUM1 ($P < .002$). TMD1A illness duration was associated with lower (1) urinary metabolite concentrations and (2) succinic acid and combined glutamine + glutamic acid levels, suggesting a progressive depletion of metabolite reserves.* ***Conclusion:*** *In TMD1A patients, total amino acid excretion was positively correlated with pain intensity and negatively correlated with illness duration, which indicated that illness duration was associated with a different set of metabolic anomalies compared with those identified for pain intensity.*

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Temporomandibular disorder (TMD) patients report muscle pain as their major complaint.[1] However, an earlier report from our research group[2] has indicated that, compared with age- and sex-matched controls, these patients also report fatigue, muscle weakness, muscle fatigue, and an increased prevalence of multi-organ symptoms involving musculoskeletal, cardiac, gastrointestinal, and genitourinary systems.[2] These patients reported a high incidence of infective events at pain onset, accompanied by a similar increased prevalence of pain in their sexual partners.[2] No

evidence for a psychologic or depression/somatization origin was found, but patients did report increased somatic symptoms and cognitive disturbances.[2] These data suggested that a generalized systemic change occurs in chronic muscle pain patients and that it may involve pathogens.

Chronic muscle pain patients who seek treatment are predominantly female (female:male ratio varies between 3.5:1 and 4:1[3,4]). Pullinger et al[3] found that, while there was a similar sex prevalence of symptoms, females had the more severe pain symptoms. Sixty-eight percent of patients with palpable muscle tenderness and 88% of patients with multiple-site palpable tenderness were female. Similarly, 78% of patients with palpable temporomandibular joint (TMJ) tenderness, and 85% of those with moderate to severe TMJ tenderness, were female. Thus, female chronic muscle pain patients have increased palpable muscle pain and more severe pain responses than males. Pain intensity was the major factor that initiated their treatment seeking.

Amino Acids in Polysymptomatic Pain/Fatigue Patients

It has been shown[5,6] that total serum amino acid levels, particularly proline, serine, tryptophan, and histidine, were reduced in a cohort of patients with fibromyalgia, and there was an anomaly in tryptophan membrane transport with concurrent reductions in serine and histidine. Further studies of urinary excretion of metabolites in polysymptomatic chronic fatigue (CFS) patients indicated changes in urinary excretion patterns, which suggested an alteration in biochemical homeostasis involving increased breakdown of muscle proteins (proteolysis).[7–9] The increases in pain intensity in these patients were primarily associated with reductions in urinary excretion of serine and phenylacetic acid, as well as increases in aspartic acid and a marker compound coded "CFSUM1." Aspartic acid may act as an excitatory amino acid and be associated with N-Methyl-D-Aspartate (NMDA) receptor activation and the initiation of persistent nociceptive (hyperalgesia) responses in chronic pain.[10] This suggests that increases in excitatory amino acids may be part of the proteolytic process that facilitates hyperalgesia and enhances pain intensity.

In patients presenting with fibromyalgia, CFS, and depression, altered hormonal excretion responses have been identified,[11,12] which involve anomalies in prolactin, growth hormone, and cortisol excretion.[11] These hormones regulate protein turnover in many cells including the liver, adipose tissue, and muscle,[13] and may be linked to the alterations in amino acid homeostasis reported in CFS and fibromyalgia.[5–9] Thus alterations in proteolysis may reflect changes in both central hormone production as well as cytokine-mediated changes.[12–14]

Proteolysis

Trauma and pathogen-induced cytokine-mediated alterations in metabolism lead to proteolysis,[12–14] which increases the availability of amino acids. Catabolism may induce cytoplasmic proteins (nonfibrillar proteolysis) or structural fibrillar proteins—actin, myosin (fibrillar proteolysis)—in muscle and other tissues. Nonfibrillar proteolysis is associated with increased release of tyrosine as a major constituent of cytoplasmic protein, while fibrillar proteolysis involves increased release from muscle fibers of the actin component, 3-methylhistidine.[14] It has been reported[15] that the measurements of the urinary output of tyrosine (nonfibrillar) and 3-methylhistidine (fibrillar) may be used to assess these 2 processes.

Muscle fibrillar proteolysis requires activation of calcium-dependent proteases and is independent of the nonfibrillar processes.[13] The nonfibrillar response[13–19] is influenced by many factors, including insulin, glucagon, growth hormone, variation in cell volume, cyclic AMP, heavy metals, and cytokines, as well as variations in specific amino acids (leucine, tyrosine, phenylalanine, glutamine, proline, methionine, tryptophan, histidine, alanine). The amino acid leucine is the major influencing factor in nonfibrillar proteolysis in liver, muscle, and adipose tissue, and has been reported[16] to have a distinct control over RNA degradation. As a result of these data,[13–19] it appears that: *(a)* proteolysis plays a significant role in the development of chronic muscle pain, and *(b)* it may be sustained by altered homeostasis in response to an as-yet unidentified activator and/or a depletion of regulator substrates such as leucine. This up-regulation of amino acid availability may also provide the basis of development of hyperalgesia by increasing excitatory amino acid availability.

In view of these considerations, the following hypothesis was formulated: Chronic muscle pain conditions are characterized by repetitive acute exacerbations manifested as increases in pain intensity that are associated with the release of amino acids and other metabolites. Increasing illness duration leads to an increase in pain distribution, symptom severity, and the development of symptoms within other tissues or organ systems.

This study aimed to address the hypothesis in part by determining whether the duration of chronic pain in TMD patients is associated with a net depletion of amino acids, and whether this is a distinct process from the changes associated with pain intensity.

Methods

Patient Selection

Forty-six patients sequentially presenting for orofacial pain management were selected on the basis of muscle pain as defined by the research diagnostic criteria/TMD (RDC/TMD1A) and not having pain associated with TMJ arthritis, sinusitis, dental nerve, or vessel pathology, or any other disease associated with pain.[2] These patients were designated as the TMD1A group and were included in the study group as previously described,[2] based upon:

- Features consistent with the RDC/TMD1A criteria[1]
- A rating of pain intensity on a visual analog scale ([VAS] range from "no pain" to "worst imaginable") for the 2 weeks prior to consultation
- The presence of palpable muscle pain in the reported pain areas of the face, head, and neck
- The pain being present on more than 50% of days during the 3 months immediately preceding consultation

Forty-one age- and sex-matched control subjects were identified (including relatives of the pain patients and unrelated subjects). Control subjects were included if they had no pain report on a VAS in the 2 weeks prior to consultation, did not give a history of chronic pain, and had not required clinical advice or treatment for chronic muscle pain in the previous 12 months, as previously described.[2] Acute pain associated with trauma during the preceding 12 months was not an exclusionary criterion. Each patient and control subject provided informed consent in accordance with ethics requirements (Universities of Sydney and Newcastle) and was assessed by 1 clinician (NMcG).

All medications being taken by the patients were recorded and all subjects were asked to cease all medications apart from antidepressant and analgesic medications. Four study subjects were taking analgesics (3 TMD1A, 1 control), and 1 TMD1A patient was taking antidepressant medication. The single control patient who reported taking analgesics had detectable acetaminophen in her urine and examination of her symptom profile revealed that she reported headache and abdominal pain but no musculoskeletal pain symptoms. No patients had taken antibiotics within the previous 4 weeks.

Questionnaires

All subjects completed a Collaborative Pain Research Unit (CPRU) questionnaire and a Hopkins Symptom Check List-90-Revised (SCL-90-R).[2] On completion, the questionnaires were checked by the clinician (NMcG) and the patients were questioned to confirm their answers. The symptom incidence index was calculated as the number of positive responses to 48 symptoms from within the CPRU, as previously reported.[2]

Confirmation of Muscle Pain

Patient-reported muscle pain was confirmed by: *(1)* palpation of superficial facial, neck, and shoulder muscles; *(2)* a VAS (assessing muscle pain intensity); and *(3)* the SCL-90-R responses to questions 1 (headaches), 12 (chest pain), 27 (low back pain), and 42 (muscle soreness), as previously described.[2]

Calculation of Body-Pain Distribution Index

A body-pain diagram was divided into 10 areas: face, TMJ, head, neck, shoulder, anterior chest wall, abdomen, back, arm, and leg. The prevalence of reporting of pain in these areas was calculated to give an indication of the body pain distribution (range 0 to 10).

Urine Specimens and Gas Chromatography Mass Spectrometry (GCMS) Identification

Each study subject collected a first-morning urine specimen on the day of the second visit. The urine was refrigerated (not frozen) and processed within 24 hours of collection.[7] Following centrifugation of a 10 mL aliquot at 1,500 rpm for 5 minutes at 4°C, 200 μL was freeze-dried in a derivatization tube for 18 hours. The freeze-dried urine material was then reacted to form the N(O,S)-heptafluorobutyryl-isobutyl (HFB-isobutyl) derivatives for analysis by a Hewlett Packard GCMS (5971A mass selective detector).[7]

Thirty-six urine peaks were selected for examination after a linear detector response was confirmed by the mass spectrometer. This is an extended list of metabolites from that used in the

previous studies.[7] Where possible, the peaks were identified by HP-UX Chemstation computer search of user-generated reference libraries (incorporating retention indices and mass spectra) and the WILEY Database. The original peaks were numbered 1 to 28 in order of retention time and those peaks that could not be identified were allocated a reference code (either UM or CFSUM) as previously outlined.[7] The additional peaks were allocated an alphabetic suffix (eg, UM15a) to conform to the retention time peak allocation method previously described.[7] Relative abundance and qualitative analyses of the urine metabolites were obtained as previously described.[7–9] The area of each peak was recorded and percentage relative abundance of the 36 peaks was calculated for further analysis. Evaluation of the relative abundance data enabled assessment of metabolite changes independent of variations in urine volume. The concentration (mmol/L) of each metabolite was calculated from the peak areas using an external standard strategy. Separate GCMS were performed with known quantities of metabolite and then used to calculate the peak area in the samples.

To enable evaluation of concentration data for the 9 unidentified urinary metabolites, the coded peaks (in addition to 3 identified compounds for which authentic standards could not be readily obtained, see Table 1) were expressed as alanine-equivalent mmol/L concentrations. Quantitative urine analysis usually involves the collection of 24-hour urine samples and the assessment of peak area expressed against urinary 3-methylhistidine (or creatinine) concentration. A first of the morning urine sample was considered preferable in this study to minimize dietary influence on urine composition, standardize collection procedures, and maintain consistency with previous investigations.[7–9] The peak areas were not assessed against urinary 3-methylhistidine (or creatinine) concentration, as a previous study identified that the levels of 3-methylhistidine were correlated with the excretion of certain amino and organic acids as well as symptom severity scores.[7] These associations were different between the control and patient groups.

Statistical Analysis

The percentage composition urine data were arcsine-transformed before analysis while the concentration data were cube root-transformed to improve normality and linearity. Subject characteristics were assessed by chi-square probability and Student *t* tests. Symptom indices and metabolites were compared by the use of Student *t* tests, Spearman rank-order correlation, discriminate function and multiple regression analyses. These data were processed by the use of Access 2000, Excel 2000, and Statistica.

Results

Study Groups

Of the 46 TMD1A patients and 41 control subjects, 43 and 40, respectively, completed all questionnaires and examination requirements as previously reported.[2] From these study subjects, 14 additional TMD1A patients and 6 additional control subjects were excluded from this study, as urine samples were either not obtained (n = 16) or laboratory processing problems occurred (n = 4). The remainder comprised the study (TMD1A) (n = 29) and control (n = 34) groups: There were no statistically significant differences in the subject characteristics: age (TMD1A = 38.2 ± 13, control = 35.0 ± 13); gender characteristics (TMD1A = 83% female, control = 68% female); marital status (TMD1A = 48% married, control = 47% married).

The symptom and metabolic homeostasis anomalies observed between the TMD1A and control patients have been reported.[2] This investigation focuses on examining the features associated with both TMD1A illness duration and pain intensity, and correlation analyses were performed. The key illness parameters in the TMD1A patients included: illness duration (5.0 ± 1.2 years [range 2 to 9 years]); symptom prevalence (25.5 ± 11.3 symptoms/subject [range 0 to 48]); pain intensity (5.4 [95% confidence limits: 4.5 to 6.3], [VAS score]); and body pain distribution (6.3 ± 2.4 body areas). The control group did not report parameters of illness duration, pain (VAS), or body regions and showed a much lower SCL-90-R symptom prevalence of 5.2 ± 5.0 symptoms/subject compared with the TMD1A patients ($P < .001$). Table 2 shows Spearman rank-order correlation analyses between these variables. As illness duration increased, the number of symptoms reported increased ($P < .005$), as did the number of body areas of pain ($P < .05$), suggesting a progression of illness complexity. Pain intensity, however, was not correlated with illness duration, symptom prevalence, or body pain distribution. There was no difference in any of these parameters in the patients who reported either a sudden (n = 9) or gradual onset (n = 15; data not shown).

Table 1 Urine Excretion Data Measured in TMD1A Patients (n = 29) and Control Subjects (n = 34)

		Concentration (μmol/L)			% Relative Abundance†		
Peak no.	Metabolite	Control Mean (SE)	TMD1A Mean (SE)	P*	Control Mean (SE)	TMD1A Mean (SE)	P*
1	Ethanolamine	1,412 (199)	1,302 (177)	NS	1.68 (0.15)	1.76 (0.22)	NS
2	Serine	1,647 (282)	1,315 (195)	NS	7.21 (0.50)	4.65 (0.32)	< .001
3	Alanine	3,440 (491)	2,573 (378)	NS	4.77 (0.34)	3.25 (0.37)	< .003
4	Glycine	5,441 (639)	4,577 (628)	NS	19.64 (1.43)	13.39 (1.27)	< .002
5	β-Alanine	191 (45)	280 (62)	NS	0.63 (0.10)	0.86 (0.15)	NS
5a	Valine	157 (30)	123 (17)	NS	0.95 (0.08)	0.66 (0.07)	< .008
5b	Threonine	350 (69)	282 (39)	NS	1.64 (0.14)	1.15 (0.11)	< .02
6	β-Aminoisobutyrate‡	369 (76)	524 (127)	NS	0.53 (0.10)	0.70 (0.16)	NS
7	Leucine	70 (20)	13 (5)	< .001	0.32 (0.04)	0.08 (0.02)	< .001
8	Phenylacetic acid‡	1,179 (184)	1,421 (268)	NS	1.83 (0.27)	1.60 (0.32)	NS
9	Proline	449 (76)	370 (53)	NS	2.01 (0.20)	1.53 (0.18)	NS
10	Succinic acid	21,140 (3,550)	28,836 (6,031)	NS	1.38 (0.17)	1.45 (0.29)	NS
10a	S-methylcysteine	272 (41)	407 (97)	NS	0.31 (0.06)	0.36 (0.08)	NS
11	Asparagine	1,977 (234)	2,411 (330)	NS	1.66 (0.22)	1.48 (0.22)	NS
11a	Hydroxyproline	38 (7)	33 (5)	NS	0.48 (0.05)	0.47 (0.07)	NS
12	CFSUM1‡	1,904 (463)	4,386 (889)	< .008	2.56 (0.52)	4.73 (1.07)	< .03
13	UM13‡	33 (12)	20 (5)	NS	0.05 (0.01)	0.03 (0.01)	NS
13a	UM13a‡	105 (25)	201 (109)	NS	0.15 (0.03)	0.24 (0.11)	NS
14	UM14‡	91 (30)	72 (16)	NS	0.14 (0.04)	0.09 (0.02)	NS
15	UM15‡	9 (5)	33 (23)	NS	0.01 (0.01)	0.03 (0.01)	NS
15a	UM15a‡	316 (51)	525 (132)	NS	0.49 (0.07)	0.73 (0.18)	NS
15b	Acetaminophen‡§	6 (5)	375 (207)	< .03	0.01 (0.01)	1.04 (0.90)	NS
16	Aspartic acid	500 (101)	516 (80)	NS	1.87 (0.12)	1.73 (0.20)	NS
17	UM17‡	91 (19)	113 (31)	NS	0.13 (0.03)	0.13 (0.04)	NS
18	Phenylalanine	124 (19)	138 (21)	NS	0.94 (0.07)	0.94 (0.10)	NS
19	Ornithine	55 (11)	61 (16)	NS	0.78 (0.11)	0.92 (0.14)	NS
20	Glutamic acid	2,976 (581)	2,606 (304)	NS	11.86 (0.86)	9.35 (0.74)	< .05
21	Lysine	302 (88)	289 (88)	NS	3.50 (0.64)	4.27 (1.02)	NS
22	Tyrosine	25 (7)	41 (9)	NS	0.32 (0.06)	0.83 (0.20)	< .02
23	1-Methylhistidine	66 (42)	134 (75)	NS	0.54 (0.31)	2.68 (0.93)	< .02
24	3-Methylhistidine	135 (28)	141 (29)	NS	1.73 (0.23)	2.05 (0.34)	NS
25	Hippuric acid	6,929 (1,485)	9,037 (2,109)	NS	20.14 (2.58)	23.47 (3.41)	NS
26	Aconitic acid	1,216 (154)	1,544 (184)	NS	7.01 (0.77)	7.43 (0.69)	NS
26a	Citric acid	4,411 (2,110)	9,072 (3,808)	NS	1.01 (0.32)	2.92 (0.79)	< .04
27	UM27‡	1,444 (584)	1,500 (404)	NS	1.26 (0.40)	2.13 (0.38)	< .05
28	UM28‡	845 (509)	710 (138)	< .006	0.45 (0.21)	0.87 (0.15)	< .001
1-28	Total metabolites	59,714 (8,365)	75,979 (10,172)	NS			
Discriminate function analyses		Wilks λ = .334, F = 7.53, P < .001			Wilks λ = .322, F = 7.95, P < .001		
Forward stepwise models							
Discriminate variables		(1) leucine P < .001 (2) tyrosine P < .007 (3) CESUM1 P < .04			(1) leucine P < .001 (2) tyrosine P < .002 (3) serine P < .05		

*Statistical methods: Student *t* test and discriminate function analyses. α = P < .05; NS = not statistically significant; SE = standard error.
†Calculated from arbitrary area unit detector response values. ‡Expressed as alanine equivalents as described in Methods. Wilks λ = the ratio of the determinant of the within-groups variance/covariance matrix over the determinant of the total variance covariance matrix; §Not included in the discriminant function analysis.

Group Differences in Urinary Excretion Patterns

Thirty-six urinary metabolites were detected and measured. The mean relative abundance and concentrations of urinary metabolites analyzed are summarized in Table 1, together with the results of multivariate and univariate statistical analyses. Forward stepwise discriminate function analyses of the concentration and relative abundance data (Table 1) indicated that the TMD1A patients had different urinary excretion profiles compared with the control subjects ($P < .001$, overall classification accuracy > 90% for both models). Leucine excretion was lower in the TMD1A group (Table 1, *t* test $P < .001$), and was the primary factor differentiating the 2 study groups by discriminate analysis ($P < .001$). The other primary discriminate factors in the concentration analysis were

tyrosine ($P < .007$) and CFSUM1 ($P < .04$). In the relative abundance analysis, the second and third primary discriminate factors were tyrosine ($P < .002$) and serine ($P < .05$).

Student *t* test analyses of both the urine concentration and relative abundance data also indicated that urinary excretion of several metabolites were different for TMD1A patients compared with controls (Table 1). The concentrations and relative abundance of CFSUM1 was elevated in the TMD1A group as shown in Table 1. The concentration and relative abundance of urinary tyrosine was also higher in the TMD1A group (although not statistically significant in the concentration analysis). The tyrosine:leucine ratio was elevated in the TMD1A patients (mean ± SE; TMD1A = 7.21 ± 1.91, control = 1.13 ± .23; $P < .001$; all subjects with 0 values deleted), which indicates a deregulation in protein metabolism. In the TMD1A patients, the relative abundance of 1-methylhistidine, citric acid, and UM27 were elevated, whereas serine, alanine, glycine, valine, threonine, and glutamic acid were reduced (Table 1).

Acetaminophen (paracetamol) was also detected in higher concentrations in the urine from TMD1A patients as might be anticipated. Since acetaminophen is an externally administered analgesic, it was omitted from the multivariate analyses reported above. Inclusion of this compound in the discriminate function analysis slightly improved the model separation statistics, but did not alter the order or the statistical significance of the primary discriminate variables contributing to the separation models (Table 1). The concentrations of acetaminophen in the urine were not associated with any of the primary discriminate, regression, or *t* test variables that differentiated between the TMD1A and control groups.

Differences in Urinary Metabolites in Relationship to Pain Intensity and Illness Duration

Two types of analyses were undertaken. These were assessments of associations between pain intensity and illness duration in: *(a)* the whole study cohort (the results of which also incorporate differences between the TMD1A and control groups), and *(b)* within TMD1A group (the results only show variations in the parameters within the pain group). Failure to assess these inter- and intragroup differences may not allow the changes in chemistry in relationship to the pain intensity and illness duration to be identified.

Total Cohort Analyses. Multiple regression analysis was applied to the data from the total study cohort to determine whether the increases in pain intensity score and illness duration observed in the TMD1A group were directly correlated with changes in urine excretion profiles (acetaminophen omitted). Analyses of both the concentration and relative abundance data showed strong correlations between pain intensity and illness duration and changes in urinary excretion of metabolites (all 4 assessments $P < .001$; Table 2). The primary concentration and relative abundance correlates for both pain intensity and illness duration was leucine. Alterations in homeostasis reflected by urinary excretion patterns were, therefore, strongly associated with both pain intensity and illness duration.

Univariate analyses of the associations between pain intensity and illness duration are shown in Table 2. Pain intensity and illness duration were both negatively correlated with leucine and positively correlated with UM28 and CFSUM1 in both relative abundance and concentration analyses. Pain intensity was positively correlated with the total urinary metabolites and 8 metabolites in the concentration analysis and positively correlated with 3 metabolites and negatively correlated with 6 metabolites in the relative abundance analysis. In the concentration analysis, illness duration was positively correlated with 3 metabolites. In the relative abundance analysis, illness duration was positively correlated with 4 metabolites and negatively correlated with 7 metabolites. Minor differences in the metabolites were found between pain intensity and illness duration. Both pain intensity and illness duration were positively correlated with the tyrosine:leucine ratio. Figure 1 shows the relative abundance of tyrosine and leucine in relation to pain intensity.

TMD1A Cohort Analyses. Multiple regression analysis was applied to the data from the TMD1A group to determine whether the increases in pain intensity score and illness duration observed in the TMD1A group were directly correlated with changes in the urine excretion profiles (acetaminophen omitted), and to assess if they differed from those observed when assessing the whole study group. Analyses of both the concentration and relative abundance data showed strong correlations between pain intensity and illness duration and changes in urinary excretion of metabolites (all 4 assessments $P < .005$; Table 2). All 4 analyses were associated with different primary metabolites. The metabolites that correlated with pain intensity and illness duration were quite different from those found for the whole study cohort. Pain intensity was negatively correlated with leucine and positively correlated with

Table 2 Summary of the Urinary Excretion of Metabolites Significantly Correlated* with Pain Intensity or Illness Duration Scores within the (a) Whole Study Group (n = 63) and the (b) TMD1A Group (n = 29)

	Pain Intensity				Illness Duration			
	mmol/L		%		mmol/L		%	
	r	*P*	*r*	*P*	*r*	*P*	*r*	*P*
(a) Whole study group								
Total metabolites†	0.27	< .04						
Tyrosine:leucine ratio†	0.59	< .001			0.64	< .001		
Positive correlations								
UM28	0.57	< .001	0.54	< .001	0.47	< .001	0.55	< .001
CFSUM1	0.40	< .002	0.32	< .02	0.25	< .05	0.26	< .04
UM27	0.30	< .02	0.30	< .02			0.35	< .006
Aconitic acid	0.31	< .02						
1-Methylhistidine	0.30	< .02			0.35	< .005		
Tyrosine	0.30	< .02					0.28	< .03
Citric acid	0.29	< .03						
β-Alanine	0.27	< .04						
Negative correlations								
Leucine	–0.63	< .001	–0.68	< .001	–0.61	< .001	–0.59	< .001
Serine			–0.41	< .001			–0.43	< .001
Glycine			–0.37	< .004			–0.38	< .002
Alanine			–0.34	< .007			–0.35	< .005
Threonine			–0.29	< .03			–0.35	< .006
Valine			–0.26	< .05			–0.33	< .008
Glutamic acid							–0.29	< .02
Multiple regression analysis								
	R^2 = 0.745		R^2 = 0.704		R^2 = 0.636		R^2 = 0.738	
	F = 13.28		F = 10.81		F = 6.83		F = 7.29	
	P < .001		*P* < .001		*P* < .001		*P* < .001	
First correlates								
Leucine	*P* < .001		*P* < .001		*P* < .001		*P* < .001	
(b) TMD1A group								
Total metabolites†	0.40	< .03			–0.43	< .02		
Tyrosine:leucine ratio†	0.55	< .002						
Positive correlations								
Aspartic acid	0.55	< .002			–0.39	< .05		
Glutamic acid	0.55	< .002			–0.43	< .04		
Serine	0.49	< .008						
Ethanolamine	0.48	< .01						
3-Methylhistidine							0.51	< .005
Aconitic acid							0.38	< .04
Negative correlations								
Leucine	–0.49	< .008	–0.45	< .02				
Succinic acid					–0.56	< .004	–0.40	< .03
Multiple regression analysis								
	R^2 = 0.887		R^2 = 0.824		R^2 = 0.912		R^2 = 0.982	
	F = 5.10		F = 5.40		F = 10.30		F = 22.90	
	P < .005		*P* < .005		*P* < .001		*P* < .001	
First correlates								
Aspartic acid	*P* < .001							
Leucine			*P* < .001					
Succinic acid					*P* < .001			
3-Methylhistidine							*P* < .001	

*Statistical methods: Spearman rank-order correlation and multiple regression analyses. α = *P* < .05; *r* = Spearman rank-order correlation; R^2 = total variance of the dependent variable as explained by the independent variables (maximum 1.0).
†Not included in the multiple regression analysis.

the total metabolites and 4 amino acids in the concentration analysis; it was negatively correlated with leucine in the relative abundance analysis. Conversely, illness duration was negatively correlated with the total metabolites and with 1 metabolite in the concentration analysis; it was positively correlated with 2 metabolites and negatively with succinic acid in the relative abundance analysis. Alterations in homeostasis reflected by urinary excretion patterns were, therefore, strongly associated with both pain intensity and illness duration and were quite different from those seen in the whole study cohort.

Detailed data analysis revealed that the longer the duration of TMD1A illness, the lower the total level of urine metabolites excreted (Table 2, Fig 2). In contrast, the higher the pain intensity, the higher the level of total urine metabolites excreted (Table 2, Fig 3). The longer illness duration was correlated with diminished concentration outputs of succinic acid and combined glutamine + glutamic acid and an increase in the relative abundance of 3-methylhistidine and aconitic acid. Higher levels of pain intensity were associated with diminished levels of urinary leucine and increased urinary levels (concentration analysis) of excitatory amino acids (aspartic acid, Fig 4; combined glutamine + glutamic acid, Fig 5) as well as serine and ethanolamine (Table 2).

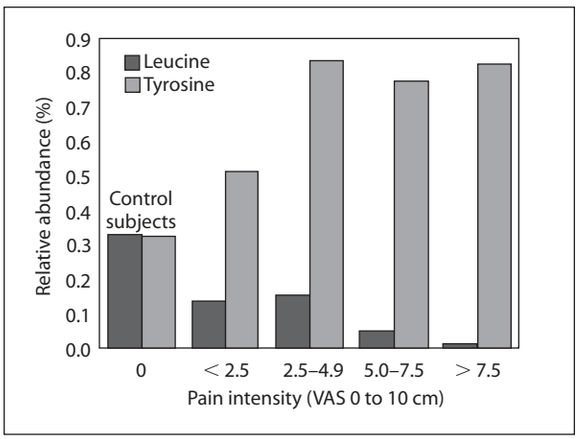

Fig 1 Variations in the relative abundance of tyrosine and leucine in the urine of study subjects divided on the basis of pain intensity scores (controls VAS = 0; TMD1A patients VAS = > 0).

Discussion

The excretion of urinary metabolites assessed in this study were analyzed by 2 different methods: *(1)* the concentration, in mmol/L, of the metabolites in a measured volume of urine; and *(2)* the relative abundance (percentage distribution) of those metabolites within the total metabolites found in the sample. These methods were used since trauma-induced or cytokine-mediated changes may be associated with an increase in pain and also a change in renal function.[20,21] Trauma-induced or cytokine-mediated responses are able to induce an aminoacidemia as well as an aminoaciduria,[21] which may alter the results if only the concentration analysis was used. This can be demonstrated, as the concentration data in Table 1 revealed only 4 differences, while the relative abundance data revealed 13 differences. This shows that potentially significant data can be found by assessing the data in these different ways.

Similarly, the data were also assessed by comparing the whole study cohort data with the TMD1A cohort data. This allowed a better understanding of the biochemical processes involved in the development of TMD, pain intensity, and illness duration. For example, while there were no changes in total metabolite excreted between the TMD1A and control groups, the total metabolite levels were positively correlated with pain intensity and negatively correlated with illness duration in the TMD1A group. This suggests that different processes are involved. Analysis using the total cohort reflects changes involved in the development of TMD, while analysis within the TMD1A group is linked with the variation in chemistry in relation to pain intensity and illness duration, reflecting the altered biochemical status of subjects with TMD. Failure to analyze these differences may fail to identify the actual changes in chemistry, potentially leading to inappropriate interpretations and conclusions.

This study has identified a strong association between chronic RDC/TMD1A muscle pain and alterations in urinary excretion of amino acids. Compared with controls, the TMD1A group had reduced excretion of leucine, and the relative abundance of alanine, glycine, serine, valine, and glutamic acid + glutamine. Leucine was the primary variable that predicted: *(a)* the difference between the TMD1A and control groups, *(b)* pain intensity and illness duration in the whole group data, and *(c)* pain intensity within the TMD1A patient cohort. Therefore, a fall in leucine appears to be important for the development of chronic pain in this cohort and correlated with pain intensity. Importantly, leucine and many of the other amino acids low in the TMD1A patients are known to

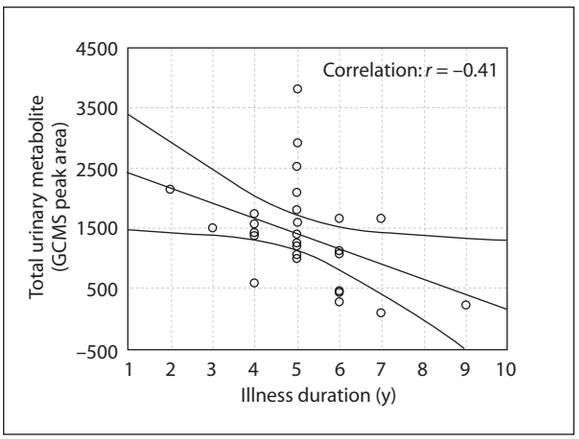

Fig 2 Variations in the total urinary metabolite and illness duration (years) in TMD1A patients. The center line is the correlation line and the outer lines are the 95% confidence limits.

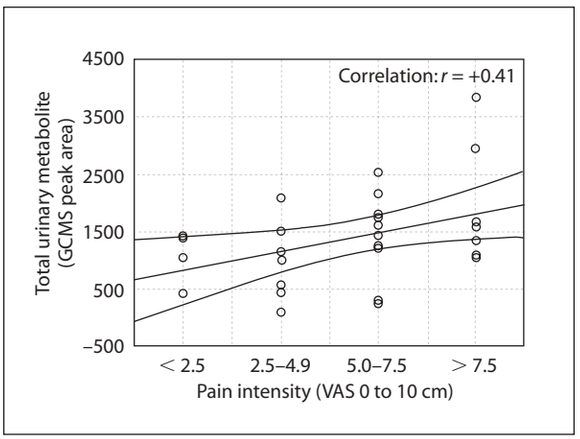

Fig 3 Variations in the total urinary metabolite and pain intensity (VAS: 0 to 10 cm) in TMD1A patients. The center line is the correlation line and the outer lines are the 95% confidence limits.

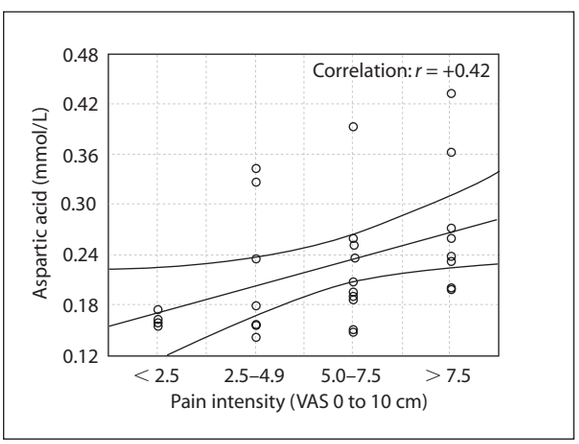

Fig 4 Variations in the mean concentration of the excitatory amino acid, aspartic acid, and pain intensity (VAS: 0 to 10 cm) in TMD1A patients. The center line is the correlation line and the outer lines are the 95% confidence limits.

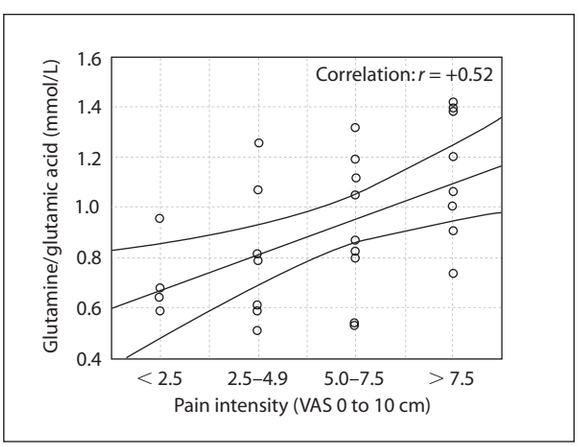

Fig 5 Variations in the mean concentration of the combined excitatory amino acid, glutamine + glutamic acid, and pain intensity (VAS: 0 to 10 cm) in TMD1A patients. The center line is the correlation line and the outer lines are the 95% confidence limits.

modulate proteolysis,[13,16,19] suggesting that the changes may be associated with an alteration in protein turnover. Leucine is an essential amino acid and 1 of the 3 branched chain amino acids, which includes isoleucine and valine. The branched chain amino acids are released from tissues following trauma and during cytokine-mediated events[16,19] and are involved in control of the nonfibrillar proteolytic response. This control of protein synthesis appears to be linked with insulin levels and excitation coupling with intracellular activation proteins.[22,23] The reduced leucine levels suggest that there may be a deficit in this amino acid, which might contribute to sustaining the proteolytic response resulting in the observed reduction in intracellular protein and RNA as seen in patients with fibromyalgia, CFS,[24–26] and other common muscle pain situations.[16,19,22,23] These observations are consistent with reductions in equivalent serum amino acids reported for patients with fibromyalgia,[5,6] sepsis, or following trauma,[20,21] and strongly suggest that the TMD1A patients have a reduction in protein turnover and that these changes may be involved in the development of TMD1A pain.

While the progressive fall in leucine levels was a good predictor of pain intensity in the TMD1A

patients, pain intensity was also associated with an increase in total urinary amino acid levels; this supports the possibility of a cytokine-mediated event.[16,19] Although there were no differences compared with the control group in the urinary excretion of aspartic acid, serine, and combined glutamine + glutamic acid, the TMD1A group revealed strong positive associations between urinary concentrations of these amino acids and pain intensity. Increases in excitatory amino acids in the spinal cord and central nervous system are involved in the initiation of hyperalgesia,[10,27,28] suggesting that the amino acid changes may be related to the development of hyperalgesia within the TMD1A group. The increase in pain intensity and hyperalgesia is also noted following acute and severe trauma.[21] However, the total urinary metabolite output between the TMD1A and control groups was not different and appears to be the result of the combined effects of the fall in amino acids with illness duration and the increases with pain intensity, a combination of events that appear to mask each other when a simple group comparison is undertaken. This suggests that repetitive or sporadic increases in pain intensity appear to be associated with increases in amino acid release followed by periods of lower pain intensity and lower amino acid levels associated with whole body depletion. This is supported by the observation that there was no correlation between pain intensity and illness duration, body pain distribution, or symptom prevalence, as well as epidemiologic observations, and that there was no correlation between muscle tenderness scores and pain intensity.[29] A prolonged cycle of repetitive increases in amino and organic acid excretion is likely to result in a net reduction in total body amino acid levels (as reported in fibromyalgia syndrome subjects[5,6]) and a gradual symptom onset with a progressive increase in symptom severity and body pain distribution, as previously reported.[2] A localized area of pain at the onset of the TMD followed by an increase in severity, multiple organ involvement, and the development of a more widespread pain condition, has been observed in this patient cohort. This is also a significant finding in TMD, fibromyalgia, and CFS patients, with many patients also reporting a gradual onset of their disorder.[2,5,6] The latter is supported by the widespread pain distribution found in most chronic TMD patients,[2,30–33] and implies that a progressive series of changes is occurring in these patients.

In support of the depletion of body reserves of amino acids and the gradual development of pain and whole body symptom expression,[2,30,31] patients with the longest illness duration had lower levels of urinary metabolites and increases in 3-methylhistidine. Increases in 3-methylhistidine strongly suggest that there is an increase in degradation of muscle fibers and cytoskeletal actin[34,35] indicating the possible depletion of the nonfibrillar protein source. Also supportive of a protein turnover problem is the increase in urinary 1-methylhistidine for both pain intensity and illness duration. 1-Methylhistidine and 3-methylhistidine are components that originate from cytoskeletal actin,[34] but may also be found in urine of subjects eating meat products. In this case, the levels correlate with symptom expression and appear to indicate the degradation of cytoskeletal proteins that usually occurs in the late stage of cancer or under severe protein degradation situations. The therapeutic use of cytokine results in similar musculoskeletal pain and mood alterations noted in TMD patients,[36,37] and may be the result of the combined cytokine stimulus and the amino acid depletion. While cytokines may be the obvious stimulus to trigger these events, other influences may also be involved. Alterations in the pathways associated with cytokine activation, such as the interferon 2, 5A synthetase RNase-L pathway, can also be triggered by chemicals and intracellular viruses resulting in low intercellular RNA and protein levels.[24–26,38,39] Similarly, bacterial toxins such as staphylococci[40,41] and even hormones[42] are capable of initiating or modifying the responses. Thus a complex array of stimuli may initiate the fall in protein and the increase in protein demand suggested by the increased methylhistidine excretion with longer illness duration.

Table 3 shows the array of mechanisms that may be influenced by depletion of amino acids as a result of the disease process (a conditional deficiency state)[43] and not from a fall in dietary intake. An example is the progressive loss of combined glutamine + glutamic acid that will influence not only excitatory neurotransmission but also the increase in gastrointestinal symptoms in muscle pain patients,[2] as glutamine is a major energy source for gastrointestinal enterocytes.[44] Chronic pain may therefore be associated with a chronic conditional deficiency state for many different amino acids, which may result in significant changes in body chemistry influencing protein production, receptor expression, and neurotransmitter availability. Depletion of amino acids and the resultant increase in glucose dependence could also partly explain why some TMD patients have episodes of hypoglycemia.[45] Thus a state of conditional amino acid deficiency is likely to be involved in the gradual

Table 3 Summary of the Potential Metabolic Associations Which May Be Affected by the Various Changes in Urine Excretion Which Were Correlated with Pain Intensity or Illness Duration

Urine excretion anomaly	Interpretation/associated metabolism[13,15–19,21,23,25,26,34,35,42]
Total metabolites	Altered kidney function, catabolism
Tyrosine:leucine ratio	A measure of protein catabolism to protein synthesis
Leucine	Indicator of protein synthesis, regulator of protein catabolism
Tyrosine	Precursor for catecholamine synthesis and marker of serum protein levels
Aspartic and glutamic acids	Excitatory amino acids, nitrogen metabolism, transamination, gluconeogenesis, nitric oxide precursors via the enzymes aspartate aminotransferase and argininosuccinate transaminase
Alanine	Nitrogen metabolism, transamination, gluconeogenesis
Aconitic and citric acids	Citric acid cycle intermediates; aconitase inhibited by nitric oxide; aconitic acid important for the metabolism of iron and RNA translation
Succinic acid	Citric acid cycle intermediate and mitchondrial oxidative phosphorylation
Serine	A very important amino acid as a precursor to glycine and ethanolamine; required for the formation of tetrahydrofolate derivatives; converted to d-serine in the CNS and can activate NMDA receptors; present in the polar head group of important complex lipids as components of cell membranes
Ethanolamine	Present in the polar head group of important complex lipids as components of cell membranes; may indicate changes in membrane lipid metabolism
β-alanine	β-amino acid, degradation product of uracil, excitatory amino acid
Glycine	Inhibitory neurotransmitter used as conjugate to form hippuric acid in the liver; formation of bile salts
Threonine	Transported across the blood brain barrier where it can be converted to glycine (glycine cannot cross the blood brain barrier)
Valine	Essential branched chain amino acids
UM28, UM 27, and CFSUM1	Unknown urinary metabolites

development or progression of chronic pain and possibly many of the associated behavioral changes. Evaluation of these data suggests that the strategic provision of amino and organic acids may represent a new therapeutic intervention for chronic pain patients, as suggested in other studies.[46]

Illness duration was also associated with reduced levels of urinary excretion of succinic acid. This may have originated from a prolonged cytokine-mediated event as previously suggested. Injection of cytokines, such as interferon (IFN), has been associated with an increase in the urea cycle glucocorticoid-regulated enzyme arginino-succinate synthetase, which facilitates nitric oxide production,[47] and is also associated with inhibition of complex 1 (NADH: ubiquinone oxidoreductase) of the mitochondrial respiratory chain.[48] This mechanism is consistent with the aminoacidemia/ aminoaciduria changes as noted with pain intensity in this study. Prolonged exposure of IFN-γ and bacterial lipopolysaccharide (LPS) also inhibits mitochondrial cytochrome-c oxidase and succinate-cytochrome-c reductase activity,[49,50] which would potentially result in reduced succinic acid levels. The data from this study may be consistent with a cytokine and bacterial toxin interaction, perhaps mediated by the coagulase-negative staphylococci previously linked with TMD1A pain.[40,51] In addition, succinic acid regulates enzymes associated with gluconeogenesis and reduction in its concentration may influence glucose metabolism,[49,50] leading to hypoglycemia noted in some myalgic patients.[45] Unpublished data from our group show a positive association between fasting serum glucose levels and urinary succinate concentration in chronic pain patients, indicating that a fall in glucose is associated with a fall in urinary succinate levels. Thus the fall in available amino acids associated with illness duration appears to be associated with an increased inhibition of oxidative phosphorylation and an increased dependence of the body upon glucose as an energy source.

Muscle pain and fatigue are common host responses to "insults," while variations in central and peripheral tissue hormonal responses may lead to further variation in symptom expression. This is particularly relevant to many disease states and reflects the response pattern associated with musculoskeletal pain. It may mask the diagnosis in patients who have the same etiologic agent and a different host response and symptom expression, or who have different etiologic agents with a common host response and symptom expression. Although this study did not address these issues,

they are recognized as important for a broader understanding of chronic pain syndromes.

Clinical studies of chronic muscle pain and chronic fatigue most commonly focus on diagnoses based on the common symptoms of muscle pain (fibromyalgia) and fatigue (CFS). The diagnostic criteria do not address the heterogeneity arising from different etiologies, as may also be the case for chronic RDC/TMD1A muscle pain. A major limitation in this study is the difficulty of determining whether the changes observed are specific for RDC/TMD Type 1A pain, or are a common event found in all pain syndromes. The biochemical events specific to RDC/TMD Type 1A pain expression can only be evaluated by a series of studies that allow comparison of changes associated with each of the defined pain conditions, CFS, fibromyalgia, and TMD pain. These studies also need to evaluate the differences between potential co-morbid disease and conditions, such as lifestyle and medical drug usage, as well as the differences between the initiating stimuli and the host response.

Conclusion

Changes indicative of proteolysis were demonstrated in TMD1A patients and were directly correlated with the differences between TMD1A patients and controls as well as pain intensity. Importantly, illness duration in chronic TMD1A pain patients was not associated with pain intensity and was associated with reduction in excretion of leucine and total amino acids; this suggests a different process associated with amino acid depletion. The progressive increase in symptom complexity and severity associated with TMD1A illness duration may result from conditional deficiencies associated with the amino acid depletion and a possible increase in glucose dependence. Pain intensity was associated with subtle elevations in the urinary excretion of excitatory amino acids (aspartic acid, combined glutamine + glutamic acid), suggesting that this may be related to the development of hyperalgesia.

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