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ORIGINAL RESEARCH

Changes in Plasma Lipid Homeostasis Observed in Chronic Fatigue Syndrome Patients*

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A study of 60 CDC-defined chronic fatigue syndrome (CFS) patients and 39 age- and sex-matched controls was undertaken to determine whether there were any alterations in plasma lipid profiles from CFS patients compared with control subjects. Plasma samples were taken from fasted (12 h) subjects and the lipid components were extracted, saponified and analyzed by capillary gas chromatography-mass spectrometry (GC-MS). It was found that CFS patients had different plasma saponified lipid profiles compared with control subjects (standard discriminant function analysis $p < 0.02$*). The first and second most important factors, discriminating the CFS patients from the controls, were a decrease in* trans*-9-octadecenoic acid and an increase in octadecenoic acid, respectively. The relative abundance of* trans*-9-octadecenoic acid in the CFS patients was 0.74% compared with 0.99% in the controls (*$p < 0.02$*) and the* trans*-9-octadecenoic acid:octadecenoic acid ratio was 0.15 in the CFS patients compared with 0.27 in controls (*$p < 0.00002$*). The younger CFS patients (*≤ 24 *years old) could be differentiated from the older CFS patients (*> 24 *years old) on the basis of their lipid profiles, whereas no such differentiation could be made within the control group. These results support an organic basis to CFS and indicate that compliance with the clinical definition of CFS does not necessarily produce a homogeneous group of patients. The differential age-related changes may occur as a result of CFS or may actually represent predisposing factors leading to and sustaining CFS.*

Keywords: chronic fatigue syndrome, plasma lipids, *trans*-9-octadecenoic acid, octadecenoic acid, fatigue aetiology, fatty acids.

INTRODUCTION

Chronic fatigue syndrome (CFS) is characterized by unexplained, persistent or relapsing, chronic and debilitating fatigue lasting six or more consecutive months [1]. The diagnosis requires the exclusion of other known fatigue-related diseases and compliance with a clinical definition [1]. No single aetiological agent has yet been found but this may be the result of the heterogeneity of the patient groups studied under the guise of the current

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definitions. Some investigators have suggested that CFS results from a common host response to a heterogeneous group of aetiological agents involving immune and neural function. The extensive range of symptoms relating to fatigue and pain reported by CFS patients suggests alterations in metabolism and homeostasis, and an extensive evaluation of the molecular basis of the syndrome and its potential subgroupings is therefore required. Changes in serum and cerebrospinal fluid monoamines [2], as well as an alteration in the hypothalamic-pituitary-adrenal axis, have been reported in American cohorts of CFS patients [3]. Significant anomalies in urinary excretion strongly associated with symptom incidence [4] have also been noted in an Australian cohort of CFS patients. These molecular data may prove useful in the future development of objective diagnostic criteria and may also allow differentiation between various subgroups of this potentially heterogeneous group of subjects.

Recent evidence indicates that 2–5A synthetase RNAase L activity is upregulated in patients with CFS [5], suggesting ongoing viral infections or autoimmune disease. In this context, treatment with the antiviral agent Poly(1)-Poly(C_{12}U) results in improvement of the symptoms of CFS subjects [6]. Although the literature does not support a common viral aetiology (reviewed in [7]), these data nevertheless suggest that viral infections may be implicated in CFS. Viral infections can alter essential fatty acid metabolism leading to significant reductions in *n*-6 essential fatty acids in cell membranes [8]. A substantial improvement, particularly in the expression of myalgia, was reported in 85% of post-viral fatigue syndrome patients following supplementation with essential fatty acids [8]. Viral infections may induce changes in fatty acid metabolism which thereby alter the host response leading, to and sustaining, CFS.

If patients with CFS have alterations in lipid homeostasis, it should be possible to detect these alterations in blood plasma samples (12 h fasted) representing a biochemical "snapshot" of lipid homeostasis with minimal dietary input. To test this hypothesis, we screened saponified plasma lipid products of CFS patients and non-CFS control subjects using capillary gas chromatography-mass spectrometry (GC-MS). The aim of this study was to assess whether qualitative or quantitative changes in plasma lipid profiles occur in CFS patients compared with control subjects.

MATERIALS AND METHODS

Study Subjects

Patients were examined and diagnosed by two clinicians (M.D. and P.D.) from August 1992 to February 1994. CFS study patients (CFS group, $n = 60$, range 11–54 years) and non-CFS subjects (control group, $n = 39$, range 17–54 years) were identified according to the Centers for Disease Control (CDC, Atlanta, USA) CFS research criteria [9]. The 39 non-fatigued control subjects were recruited from healthy relatives of the patients and from unrelated volunteer subjects. No differences were found in any of the age and sex characteristics between the 2 study groups (CFS, age $\pm$ SD $= 35.7 \pm 10.6$ years, 62% female; Control, age $\pm$ SD $= 34.5 \pm 10.4$, 54% female). All subjects had given their informed consent and the project was approved by the Human Research Ethics Committee, the University of Newcastle.

Lipid Extraction of Plasma Samples

Whole blood (10 mL) was collected from fasted patients and control subjects (12 h) by venepuncture into a lithium heparin vacutainer (Becton Dickinson) at either the Environmental Medical Centre (Sydney, Australia) or the University of Newcastle (Australia). The collection of samples from patients and controls occurred on a regular basis over the sampling period and samples were processed immediately on receipt. The samples were

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chilled on ice and transported to the laboratory. Plasma samples were coded and then processed blind by the laboratory staff. Plasma was separated by centrifugation at 1600 g for 25 min at 4°C. Aliquots of plasma (1 mL) were transferred into 12 mL PTFE-lined screw-capped glass extraction tubes and butylated-hydroxy-toluene (BHT) was added as an antioxidant (100 μL, 10 gL^{-1} in methanol) [10]. Samples were stored at −20°C. Extraction was performed within 48 h after separation.

Lipophilic components were extracted from the plasma (1 mL) by sequentially adding 2 mL methanol, 2 mL hexane and 2 mL diethyl ether. Samples were vortexed for 60 s and then centrifuged at 1600 g for 10 min at 10°C in a Fisons MSE Chilspin centrifuge. The upper hexane/ether phase was transferred to a 50 mL glass pear-shaped flask. The aqueous residue was re-extracted twice more with 4 mL fresh hexane/diethyl ether (1:1). The combined hexane/ether extracts were evaporated using rotary evaporation under vacuum until approximately 1 mL of solvent remained. This extract was transferred to a PTFE-lined screw-capped glass derivatization tube with 3 × 1 mL hexane/diethyl ether (1:1) washes. The solvent was removed from the derivatization tube using a stream of high purity nitrogen gas until approximately 1 mL of the solvent remained and the sample was stored at −20°C. The sample was taken to dryness just prior to saponification which was performed within 24 h of extraction. This method resulted in better extraction recoveries and reproducibility compared with other reported protocols [11–13] and was more efficient in the removal of aqueous components.

The complex lipids in the samples were saponified by incubating the dried lipid extract with 100 μL 10% KOH in 95% (v/v) ethanol at 100°C for 2 h [14]. At the end of this period the sample was acidified with 60 μL 6 M HCl [15–17]. The sample was snap frozen using liquid nitrogen and freeze-dried for at least 30 min. Once dry, 500 μl 95% (v/v) ethanol was added and the sample was mixed by vortex for 20 s and centrifuged at 2000 rpm for 2 min in a Sorvall bench centrifuge. The ethanol fraction was transferred to a clean derivatization tube and two further aliquots of alcohol were used to extract the remaining saponified lipids. The combined ethanol extracts were allowed to dry on the freeze-drier for 2 h before derivatization was performed. The derivatized components were stored at 4°C overnight before analysis by GC-MS.

Following saponification the dry lipid samples were derivatized by adding 50 μL 2% (w/v) methoxyamine hydrochloride in pyridine (MOX) and incubated at 60°C for 30 min. *Bis*(trimethylsilyl)trifluoro-acetamide (BSTFA, 100 μL) was added and the sample was heated at 100°C for a further 60 min to form the trimethylsilyl derivatives (TMS) [12]. This method of derivatization was selected to allow the evaluation of sterols and the non-fatty acid components of complex lipids simultaneously with the fatty acids. Prior to injection into the gas chromatograph (GC), each sample was reheated at 100°C for 10 min, cooled to room temperature and the solvent was removed using nitrogen gas. Fresh BSTFA (100 μL) was added and the derivatives were heated for a further 5 min at 100°C to ensure that the entire sample was solubilized in the BSTFA. Each sample was centrifuged at 2000 rpm for 3 min in a Sorvall bench centrifuge prior to injection (0.5 μL) into the GC-MS.

Gas Chromatography-Mass Spectrometry

Derivatized sample components were separated using a Hewlett Packard 5890 series II GC and detected using a Hewlett Packard series 5971A Mass Selective Detector (MSD). The data were stored and processed on a Hewlett Packard Unix-based Chemstation. The GC was fitted with a 25 m, 0.20 mm internal diameter HP1 fused silica capillary column (film 0.33 μm) and a split/splitless injector. The column gave good separation of the *cis* and *trans* isomers of the unsaturated fatty acids. The GC was run with the following settings: injector temperature 300°C, temperature program 80–300°C at 3°C min^{-1}, with a 2 min hold at 80°C and a 10 min hold at 300°C. The MSD operated in EI mode and was set to scan from 45–650 atomic mass units every second. Where possible, the peaks were identified by

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TABLE 1. Comparison of fatty acid concentrations and relative percentage abundances measured in this study compared with literature values

Fatty acid			Control plasma mmol L^{-1}	Sera *et al.* 1994 [18] serum mmol L^{-1}	Control plasma % FA	Sera *et al.* 1994 [18] serum mmol L^{-1}
Lauric	C12:0		0.04	<0.05	0.5%	<0.7%
Myristic	C14:0		0.13	0.08–0.39	1.7%	1.1–3.6%
Palmitic	C16:0		2.20	1.21–3.2	29.4%	19.6–27.5%
Palmitoleic	C16:1	*n*-7	0.23	0.09–0.48	3.1%	1.4–3.6%
Stearic	C18:0		0.62	0.45–1.07	8.3%	7.0–11.2%
Oleic	C18:1	*n*-9	0.72	0.88–2.96	9.6%	15.7–27.2%
Linoleic	C18:2	*n*-6	1.45	1.5–3.4	19.4%	23.0–37.8%
DGLA	C20:3	*n*-6	0.77	0.06–0.24	10.3%	1.0–3.4%
Arachidonic	C20:4	*n*-6	1.33	0.31–0.84	17.8%	5.0–11.0%

HP-UX Chemstation computer search of user-generated reference libraries (incorporating retention indices and mass spectra) and the WILEY Database.™ Lipid profile patterns were generated by assessing each peak as a percentage abundance of the total peak area of all the peaks in the chromatogram. Single ion monitoring (SIM) was utilized for the analysis of *cis*-9, 12-C18:2 (m/z = 337) and *cis/trans* 9-C18:1 (m/z = 339) to achieve full resolution of these lipid components. The fatty acid and sterol data were converted to mmol L^{-1} using available standards. Where standards were unavailable, or the compound could not be fully identified, standard curves were extrapolated from the closest reference standard (e.g. linoleic acid for C18:2(a) and (b)). The data were also expressed as relative abundance values where each peak was calculated as a percentage of the total area of all the assessed peaks. Lipid profile patterns were evaluated as either a relative abundance (RA) profile or a peak area (PA) profile (and expressed as mmol L^{-1}). The fatty acid concentrations measured by this broader-based extraction and TMS-derivatization technique (which included assessment of sterols and other saponification products) generally corresponded to those ranges reported for more specific fatty acid analysis by methyl esters as shown in Table 1. The levels of DGLA and arachidonic acid were slightly higher than the reported reference range which may reflect the use of plasma instead of serum, or alternatively an improved extraction recovery/preservation by this method, or it may simply represent a variation within the Australian population mediated by diet.

Statistical Analysis

The percentage composition values for the plasma lipid components were arcsine transformed while the concentration/peak area values were log transformed prior to statistical analysis to give normal distributions and allow parametric statistical analysis. Group differences were assessed by standard discriminant function and multiple regression analyses as appropriate. Student's t-tests, Duncan's multiple range and Chi-square probability tests were used where indicated. These data were processed using Access™ (Ver. 97, Microsoft), Excel™ (Ver. 97, Microsoft) and Statistica™ (Ver. 5. 97 Edition, Statsoft, Tulsa).

Chemicals

Bis(trimethylsilyl)trifluoro-acetamide, methoxyamine hydrochloride, BHT and the fatty acid standards were obtained from Sigma Chemical Company. All other chemicals were

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obtained from BDH. All reagents used were of analytical grade quality (AnalaR) except the hexane and methanol which were Pesticide Analysis grade.

RESULTS

Saponified Lipids

The saponified lipid products were detected as TMS derivatives by GC-MS in the plasma samples and the concentration/relative abundance data are summarized in Table 2. The fatty acid components included saturated fatty acids (12:0 to 24:0), *n*-7 and *n*-9 monounsaturated fatty acids, and *n*-6 and *n*-3 polyunsaturated fatty acids. A number of polar saponification components were detected (such as phosphate, 1-and 2-glycerophosphate and glycerol) which were presumably derived from the alkaline hydrolysis of cholesterol esters, phospholipids and triacylglycerides. Five additional fatty acids were detected and are presented in Table 2 as "unassigned fatty acids". Although the chain length of these fatty acids and their level of unsaturation could be determined from their mass spectra, the position of unsaturation and the isomerism could not be determined by comparison with available reference standards. Cholesterol was the most abundant lipid saponification product measured in the plasma. Small quantities of the cholesterol precursor 5α-cholest-7-en-3β-ol (lathosterol), as well as the plant sterols, campest-5-en-3β-ol (campesterol) and sitost-5-en-3β-ol (sitosterol), were also detected in the plasma samples. The molar concentrations (mmol L^{-1}) for the fatty acids and sterols are also shown in Table 2, and these were consistent with the known reference ranges [18]. The levels measured for oleic acid were slightly lower, and the levels measured for dihomo-γ-linolenic acid (DGLA) and arachidonic acid were slightly higher than the range reported in the literature which may reflect population differences and/or the use of plasma instead of serum.

CFS vs Controls

The array of saponified lipid products measured in plasma samples provides a complex profile (Table 2) which can be assessed by multivariate analysis to compare lipid metabolism in the CFS patients with that in control subjects. Analysis of the RA data by standard discriminant function analysis indicated that the plasma lipid profiles from CFS patients were significantly ($p < 0.02$) different compared with those from controls (Table 3a). Forward stepwise discriminant analysis revealed that the first and second most important lipids for discriminating the CFS patients from the controls were *trans*-9-octadecenoic acid and octadecenoic acid, respectively, using either the RA or PA data. There was no detectable *trans*-9-octadecenoic acid in the plasma of 15 (25%) of the 60 CFS patients, whereas *trans*-9-octadecenoic acid was absent in only one (2.6%) of the 39 control subjects ($p < 0.003$). When the standard discriminant function model was used to classify individual subject profiles as either CFS or control on the basis of their lipid profile, it was able to assign subjects into their respective clinical groups with 87% accuracy (Table 3a). The same model was used to assign an additional 34 CFS patients (average age 51 $\pm$ 15, range 23–97) and 5 control subjects (average age 31 $\pm$ 11, range 23–47), which were not used in the study, to test the power of the predictive model. The discriminant function was able to assign these external subjects with a 67% accuracy.

Since *trans*-9-octadecenoic acid (reduced in CFS) and octadecenoic acid (increased in CFS) were the major discriminant lipids for differentiating CFS patients and controls, the *trans*-9-octadecenoic acid:octadecenoic acid ratio (PA data) was also calculated and compared between the two groups. The *trans*-9-octadecenoic acid:octadecenoic acid ratio in the CFS group (mean $\pm$ SD, 0.15 $\pm$ 0.11) was lower ($p < 0.000004$) than that measured in the control group (0.27 $\pm$ 0.15).

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TABLE 2. The relative abundance and absolute amount of the saponified lipid products detected by GC-MS in plasma extracts from control subjects ($n = 39$) compared with the CFS patient ($n = 60$) groups

Saponification product	Common name	Control RA%	CFS RA%	*t*-test *p*-value	Control mmol L^{-1}	CFS mmol L^{-1}	*t*-test *p*-value	Reference range in serum [18] mmol L^{-1}
Polar lipid fractions								
phosphoric acid	phosphate	2.78	3.17	NS				
propane-triol	glycerol	0.76	1.05	NS				
succinic acid	succinate	0.04	0.05	NS				
glyceric acid	glycerate	0.18	0.21	NS				
2-glycero-phosphate	2-GP	2.59	2.76	NS				
1-glycero-phosphate	1-GP	2.00	2.17	NS				
Saturated fatty acids								
Dodecanoic acid (C12:0)	Lauric acid	0.07	0.08	NS	0.04	0.04	NS	< 0.05
Tetradecanoic acid (C14:0)	Myristic acid	0.62	0.69	NS	0.13	0.11	NS	0.08–0.39
Hexadecanoic acid (C16:0)	Palmitic acid	17.9	17.3	NS	2.20	1.84	NS	1.21–3.20
4-Methyl-hexadecanoic acid (4M-C16:0)		0.08	0.08	NS	0.04	0.04	NS	
Heptadecanoic acid (C17:0)		0.21	0.22	NS	0.06	0.05	NS	
Octadecanoic acid (C18:0)	Stearic acid	4.04	4.54	NS	0.62	0.55	NS	0.45–1.07
Eicosanoic acid (C20:0)	Arachidic acid	0.07	0.08	NS	0.04	0.04	NS	
Docosanoic acid (C22:0)	Behenic acid	0.13	0.14	NS	0.05	0.05	NS	
Tricosanoic acid (C23:0)		0.06	0.06	NS	0.04	0.04	NS	
Tetracosanoic acid (C24:0)	Lignoceric acid	0.11	0.11	NS	0.05	0.04	NS	
Unsaturated fatty acids								
n-7 *cis*-7-Tetradecanoic (*cis*-7-C14:1)	Myristoleic acid	0.05	0.05	NS	0.04	0.04	NS	
cis-9-Hexadecanoic (*cis*-9-C16:1)	Palmitoleic acid	1.45	1.52	NS	0.23	0.19	NS	0.09–0.48
n-9 *cis*-9-Octadecanoic (*cis*-9-C18:1)	Oleic acid	11.8	11.2	NS	0.72	0.66	NS	0.88–2.96
trans-9-Octadecanoic (*trans*-9-C18:1)	Elaidic acid	0.99	0.74	< 0.007	0.33	0.33	< 0.003	
cis-15-Tetracosenoic (*cis*-15-C24:1)	Nervonic acid	0.11	0.14	NS	0.05	0.05	NS	
n-6 *cis*-9, 12-Octadecadienoic (*cis*-9, 12-C18:2)	Linoleic acid	11.8	12.7	NS	1.45	1.40	NS	1.50–3.40
cis-11, 14-eicosadienoic (*cis*-11, 14-C20:2)		0.52	0.69	NS	0.15	0.14	NS	
cis-8, 11, 14-eicosatrienoic (*cis*-8, 11, 14-C20:3)	DGLA	0.33	0.41	NS	0.77	0.67	NS	0.06–0.24
cis-5, 8, 11, 14-eicosatetraenoic (*cis*-5, 8, 11, 14-C20:4)	Arachidonic acid	0.56	0.66	NS	1.33	1.08	NS	0.31–0.84
n-3 *cis*-11, 14, 17-eicosatrienoic (*cis*-11, 14, 17-C20:3)		0.14	0.17	NS	0.27	0.31	NS	

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Unassigned fatty acids								
C18:2(a)		1.12	1.04	NS	2.19	2.06	NS	
C18:2(b)		1.01	0.89	NS	1.90	1.84	NS	
C20:1		0.15	0.18	NS	0.05	0.05	NS	
C20:4(a)		0.29	0.38	NS	0.57	0.57	NS	
C20:4(b)		0.19	0.23	NS	0.37	0.36	NS	
Sterols								
Cholest-5-en-3β-ol	Cholesterol	37.7	36.2	NS	4.13	3.51	< 0.04	3.0–5.5*
5α-Cholest-7-en-3β-ol	Lathosterol	0.06	0.05	NS	0.04	0.04	NS	
Campest-5-en-3β-ol	Campesterol	0.03	0.03	NS	0.04	0.04	NS	
Sitost-5-en-3β-ol	Sitosterol	0.02	0.02	NS	0.04	0.04	NS	

Statistical Method = Hotellings T^2 test and t-test; RA Hotelling's T^2 test; $T^2 = 7,115,000,000$; $F(34,64) = 138,100,000$; $p < 0.00001$
* Pathology service reference rang.

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TABLE 3. Discriminant function analysis of the plasma lipid profile data (relative abundance data)

Standard discriminant function model	Model statistics	Discriminant metabolites (lipid saponification products)		Sensitivity/Specificity (= percentage of study subjects correctly classified by model)					Sensitivity of validation group
(a) Clinical group comparisons				CFS (sensitivity) $n = 60$		Control (specificity) $n = 39$		Total (accuracy) $n = 99$	Total $n = 39$
CFS ($n = 60$) Controls ($n = 39$)	Wilks' Lambda = 0.488 $F = 1.89$, $p < 0.014$	1) *trans*-9-octadecanoic acid 2) octadecanoic acid	control > CFS[a] control < CFS	90%		82%		87%	67%
(b) Age dependent clinical subgroup comparisons				≤ 24 years $n = 10$	> 24 years $n = 50$	≤ 24 years $n = 9$	> 24 years $n = 30$	Total $n = 99$	
a) CFS ≤ 24 years old ($n = 10$) b) CFS > 24 years old ($n = 50$) c) Controls ≤ 24 years old ($n = 9$) d) Controls > 24 years old ($n = 30$)	Wilks' Lambda = 0.160 $F = 1.48$, $p < 0.011$	1) Cholesterol 2) *trans*-9-octadecanoic acid	a < b = c = d[a] b < a < d < c	80%	92%	56%	70%	81%	

[a] Indicates significant differences between mean values by Duncan's multiple range test, $p < 0.05$

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Heterogeneity in the CFS Group

Standard logistic regression analysis indicated that age was not correlated with changes in the RA lipid profiles in either the control group or the CFS group. Similar analysis of the PA lipid data in the control group also showed no correlation. In contrast, however, the PA lipid data for the CFS group were significantly correlated with age ($R^2 = 0.76$, $p < 0.035$), where *cis*-15-tetracosenoic acid (nervonic acid, positive association), *cis*-7-tetradecenoic acid and *trans*-9-octadecenoic acid (negative associations) were the primary correlates in the regression model. These results suggest that significant alterations in lipid homeostasis occur with age in the CFS group, but not in the control group.

To assess whether the age dependent associations in lipid homeostasis lead to heterogeneity within the CFS group, the study subjects were further subdivided into those less than 25 years old ("younger CFS"), and those greater than or equal to 25 years old ("older CFS") at the time of testing. The discriminant function analysis of the RA data indicated that the 4 groups were significantly ($p < 0.011$) different by their lipid profiles (Table 3b). The primary discriminant variables were cholesterol, which was lowest in the younger CFS, and *trans*-9-octadecenoic acid, which was lowest in the older CFS patients. Analysis of the PA data gave a similar result, and the canonical plot of the study subjects by the PA discriminant function (Fig. 1) shows the effective separation of the younger and older CFS patients from the control subjects. The younger and older controls could not be separated, whereas the two CFS subgroups were well resolved, indicating that the CFS group was heterogeneous in terms of lipid homeostasis. The canonical plot demonstrates the capacity of the discriminant function model to separate control subjects from CFS patients, and to further resolve the younger CFS patients from the older CFS patients. The lipid profiles were no different between males and females in either the control or CFS groups.

DISCUSSION

The percentage composition of most of the lipid saponification products from plasma samples was mainly conserved between CFS and control groups. However, multivariate analyses indicated that the CFS group had significantly different plasma lipid profiles when compared with the control group (Table 3a). These changes were primarily associated with a reduction of *trans*-9-octadecenoic acid (elaidic acid) and an increase in octadecenoic acid (stearic acid) in the CFS patients. The corresponding *trans*-9-octadecenoic acid:octadecenoic acid ratio was also found to be reduced in the CFS patients when compared with control subjects ($p < 0.00002$) and may represent an important marker for the objective assessment of CFS. There was no detectable *trans*-9-octadecenoic acid in the plasma of 25% of CFS patients compared with only 3% of control subjects ($p < 0.003$). These results indicated that significant changes in the metabolism of *trans*-9-octadecenoic acid and octadecenoic acid occured in the CFS patients.

The onset of CFS can occur at any age, but is most frequently observed among the 12–20-year-old or the 30–40-year-old age groups. The canonical plot of the lipid profile data in Fig. 1 shows that the controls can be separated from the CFS patients who comply with defined CFS (1), and that these CFS patients can be further subdivided into younger (≤ 24 years) and older (> 24 years) subsets. Age was not associated with changes in the lipid profile in the controls, but was associated with changes in the PA lipid profiles in the CFS patients. The differential age-dependent changes may occur as a result of CFS or may actually represent predisposing factors for contracting and sustaining CFS. Either way, the data suggest a functional alteration of lipid metabolism/homeostasis in the CFS patients.

The separation, by discriminant function lipid profile models, of the controls from the younger and older subsets of CFS supports an organic basis to CFS and also indicates that compliance with the clinical definition of CFS [1] does not necessarily produce a homogeneous group of patients. Homogeneity in clinical groups is essential for

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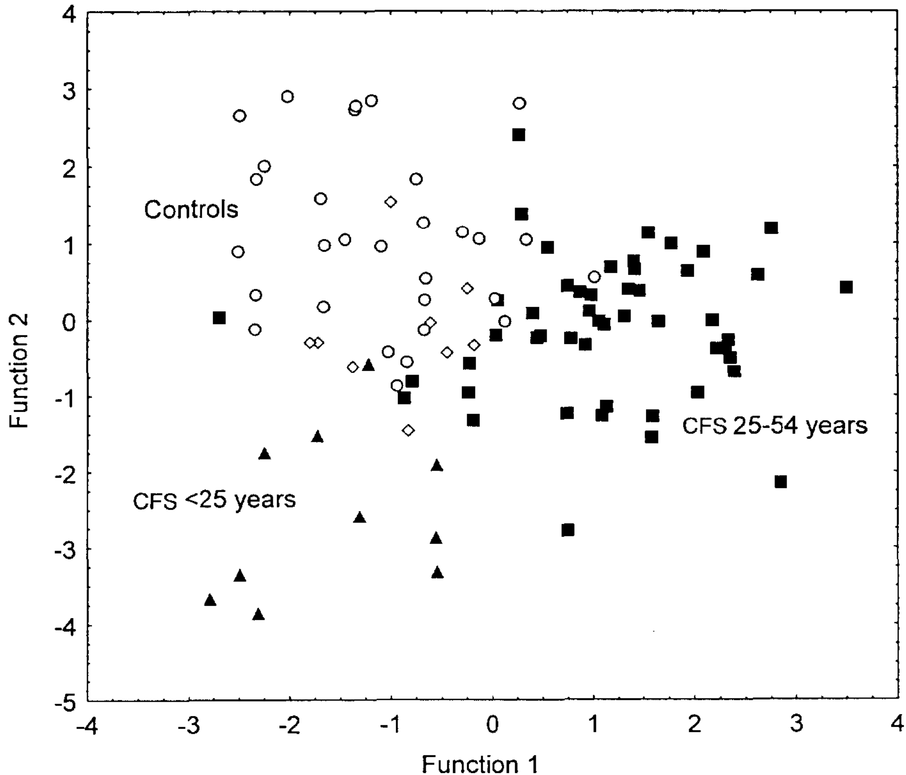

FIG. 1. The canonical plot showing the separation of the older and younger CFS and control subjects achieved by the standard discriminant function model of the saponified plasma lipids (Table 3b). (▲ CFS < 25 years; ■ CFS 25–54 years; ◇ control < 25 years; control 25–54 years).

the investigation of any specified illness and the development of effective treatment strategies. The use of plasma lipid profiles offers a potential means for objective assessment and characterization of CFS patients, and further investigation of CFS heterogeneity is therefore warranted.

The level of *trans*-9-octadecenoic acid was a major discriminant variable separating the controls from the CFS patients, as well as the younger from the older CFS subgroups. The tissue distribution of *trans*-9-octadecenoic acid in humans ranges from a low of 0.3% in brain tissue to 4.0% in adipose tissue [19]. The daily consumption of *trans*-9-octadecenoic acid in the American diet is of the order of 5–7% of fatty acids consumed (reviewed in [20]). Since alterations in serum and tissue levels of *trans*-9-octadecenoic acid are profoundly influenced by alterations in diet [21–23], the present findings could easily be dismissed on this basis. However, the membrane concentration of *trans*-9-octadecenoic acid has been shown to be highly conserved in normal situations in animal models [21], suggesting that *trans*-9-octadecenoic acid may be highly regulated in certain tissues. Although increasing dietary *trans*-9-octadecenoic acid results in increased incorporation of the lipid into rat liver and heart [22], its accumulation does not normally exceed 15% of the acyl chains in any of the lipid pools, even when dietary *trans*-9-octadecenoic acid accounts for 46% of the fatty acids supplied in the diet. The tissue accumulation of *trans*-9-octadecenoic acid in rats was at the expense of octadecenoic acid and was not associated with a change in incorporation of *cis*-5,8,11,14-eicosatetraenoic

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TABLE 4. Enzymes and metabolic functions that are altered by *trans*-9-octadecenoic acid

Enzyme or function	References
Increased activity or function with increasing *trans*-9-octadecenoic acid	
Plasma LDL (dependent upon the *cis*-9, 12-C18:2:C16:0 ratio)	[28]
Plasma cholesteryl ester transfer protein (CETP)	[27]
Glycerophosphate acyltransferase	[25]
Δ-9 desaturase (Stearoyl-CoA desaturase)	[29], [30]
Basal activity of RBC Ca^{2+} ATPase	[31]
Decreased activity or function with increasing *trans*-9-octadecenoic acid	
Plasma HDL (dependent upon the *cis*-9, 12-C18:2:C16:0 ratio)	[28]
Cyclic AMP-dependent protein kinase	[32]
Steroid 5 alpha-reductase	[33]
Carnitine acyltransferase	[34]
Δ-5 desaturase	[35]
Δ-6 desaturase	[29], [30]
Diacylglycerol acyltransferase	[36]
Monoamine oxidase	[37]
Chymase	[38]
Cardiac membrane Na^{+} K^{+} ATPase	[39]
Cardiac membrane Adenylate cyclase	[39]
Cardiac β-Adrenergic receptor numbers	[39]
Thyroid hormone stimulation of RBC Ca^{2+} ATPase	[31]

acid [23], suggesting that *trans*-9-octadecenoic acid behaves more like a saturated than an unsaturated fatty acid. In phospholipids, *trans*-9-octadecenoic acid displaces octadecenoic acid and/or hexadecanoic acid from the R1 position of phosphatidylcholine, phosphatidylethanolamine and phosphatidylinositol [24]. The data from this study suggest that there may have been a higher level of octadecenoic acid and a corresponding reduction of *trans*-9-octadecenoic acid in the R1 position of these phospholipids. Thus, while simple dietary change could be postulated as the cause of lipid changes in the present study, experimental evidence suggests that a non-dietary change in *trans*-9-octadecenoic acid metabolism may be occurring in CFS patients.

Increases in *trans*-9-octadecenoic acid have been found to inhibit cell growth and lead to increased cellular phospholipid levels [25]. In humans increasing amounts of *trans*-9-octadecenoic acid have been reported to be associated with an impairment of essential fatty acid metabolism, particularly in the C20–C22 range [26]. Dietary intakes of *trans*-9-octadecanoic acid result in a reduction of *cis*-5,8,11,14-eicosatetraenoic acid and an increase in *cis*-9,12-octadecadienoic acid in liver phosphatidylcholine and phosphatidylethanolamine [23]. Eighteen enzymes or cellular functions could be found that may be altered by changes in *trans*-9-octadecenoic acid (listed in Table 4) [25, 27–40]. It should be noted, however, that many of these changes were assessed *in vitro*, and their physiological implications have yet to be fully investigated. Several of these may be of importance in CFS as changes have been reported in carnitine acyltransferase- [40] and monoamine oxidase-associated metabolites [2].

Apart from diet, two other major possibilities exist which may offer an explanation for the reduction in *trans*-9-octadecenoic acid levels in CFS patients. Enzymes such as fatty acid omega-hydroxylase (cytochrome P-450ka2–CYP4A7), which may be activated to degrade prostaglandins (for example following an inflammation response), will also actively metabolize *trans*-9-octadecenoic acid [41] with a resultant reduction in *trans*-9-octadecenoic acid levels. It has also been reported that *trans*-9-octadecenoic acid has anti-viral activity [42] and that viruses can alter host fatty acid composition in cells [8]. Hence, specific viral infection might reduce *trans*-9-octadecenoic acid levels, thus removing

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a potential replication inhibitor to facilitate future reactivation and/or infection. These hypotheses may offer a basis for the increased susceptibility of certain patients to develop CFS after an infectious event, although other predisposing and environmental factors may also be implicated.

CONCLUSIONS

This study found that CFS patients have different plasma saponified lipid fraction profiles compared with control subjects, indicating a substantial change in lipid metabolism and homeostasis in CFS patients. These changes were primarily characterized by a reduction in *trans*-9-octadecenoic acid and a reduction in the *trans*-9-octadecenoic acid:octadecenoic acid ratio in the CFS patients. The CFS patients could be differentiated by their lipid profiles into age-based subgroups indicating lipid heterogeneity within the CFS patients.

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