Mitochondrial Function Is Related to Alterations at Brain SPECT in Depressed Patients

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ABSTRACT

Introduction: ^{99m}Tc-*d,l*-hexamethylpropylene amine oxime (^{99m}Tc-HMPAO) retention in brain is proportional to cerebral blood flow and related to both the local hemodynamic state and to the cellular content of reduced glutathione. Alterations of the regional distribution of ^{99m}Tc-HMPAO retention, with discrepant results, have been reported at functional brain imaging of unipolar depression. Since mitochondrial involvement has been reported in depressed patients, the aim of the study was to explore whether the ^{99m}Tc-HMPAO retention at single-photon emission computed tomography in depressed patients may relate to different levels of mitochondrial function.

Methods: All patients had audiological and muscular symptoms, somatic symptoms that are common in depression. Citrate synthase (CS) activity assessed in muscle mitochondria correlated strongly with the activities of three mitochondrial respiratory chain enzymes and was used as a marker of mitochondrial function. K-means cluster-

Needs Assessment

Although the literature on functional brain imaging and major depression mostly show decreased radiotracer retention implicating a general decrease of neuronal activity, discrepant results have been reported with the commonly used radiotracer hexamethylpropylene amine oxime at single photon emission computed tomography. This study aims to explore a possible effect mechanism for this phenomenon.

Learning Objectives

At the end of this activity, the participant should be able to:

- List two examples of conditions/disorders where the brain retention of hexamethylpropylene amine oxime at single photon emission computed tomography sometimes is increased.
- Give an example of a possible effect mechanism for this phenomenon.
- Appreciate the relationship between mitochondrial dysfunction and depression.

Target Audience: Neurologists and psychiatrists

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ing performed on CS grouped eight patients with low and 11 patients with normal CS. Voxel-based analysis was performed on the two groups by statistical parametric mapping.

Results: Voxel-based analysis showed significantly higher ^{99m}Tc-HMPAO retention in the patients with low CS compared with the patients with normal CS in the posterior and inferior frontal cortex, the superior and posterior temporal cortex, the somato-sensory cortex, and the associative parietal cortex.

Conclusion: Low muscle CS in depressed patients is related to higher regional ^{99m}Tc-HMPAO retention that may reflect cerebrovascular adaptation to impaired intracellular metabolism and/or intracellular enzymatic changes, as previously reported in mitochondrial disorder. Mitochondrial dysfunction in varying proportions of the subjects may explain some of the discrepant results for ^{99m}Tc-HMPAO retention in depression.

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INTRODUCTION

Alterations of the regional distribution of various radiotracers at functional brain imaging have been reported in unipolar depression. The results of a metastudy of pooled positron emission tomography (PET) and single photon emission computed tomography (SPECT) studies of unipolar depression implicated a general decrease of neuronal activity similarly affecting almost all analyzed cortical and subcortical regions, with ambiguous results for the limbic system.[1] Discrepant findings,[2-8] with increased as well as decreased retention of ^{99m}Tc-*d,l*-hexamethylpropylene amine oxime (^{99m}Tc-HMPAO) at SPECT, have been reported in unipolar depression. A summary of some recent studies can be seen in Table 1.

Liability to unipolar depression has a substantial heritable component without any evidence of a shared family environment.[9] A highly increased depression prevalence has been reported in mitochondrial disorders,[10] conditions which are oftentimes heritable. The tissues that are most affected in mitochondrial disorders are those with the highest cellular energy demands, especially brain and muscle. Signs indicating mitochondrial dysfunction have been reported in unipolar depression.[11-14] To date, there are no reports about the prevalence of mitochondrial dysfunction in depression, or concerning the issue of whether depression, per se, may affect mitochondrial function.

Intracellular trapping of the lipophilic ^{99m}Tc-HMPAO and its conversion to the hydrophilic form have been considered as the basis of retention of this tracer. The conversion from the lipophilic to the hydrophilic form has been associated to the content of reduced glutathione (GSH).[15,16] Increased GSH levels have been reported in the early stages of mitochondrial disorders.[17]

The aim of the present study was to explore the relationship between ^{99m}Tc-HMPAO retention at SPECT and mitochondrial function in a group of depressed patients in order to enlighten the issue of whether a mitochondrial involvement may contribute to the enigmatic discrepant results at ^{99m}Tc-HMPAO SPECT in unipolar depression. Mitochondrial function was assessed by the activity of citrate synthase (CS) in isolated muscle mitochondria. CS is a Krebs cycle enzyme that provides the electrons necessary for the mitochondrial respiratory chain. CS activity is considered to be a marker, even if not a direct measure, of oxidative phosphorylation reflecting the production of cellular energy, adenosine triphosphate (ATP).[18] A good correlation has been observed between CS activity and respiratory chain enzymes in normal rat tissues suggesting coordination between CS and respiratory chain enzymes, and confirming the utilization of CS activity as a marker of respiratory chain content.[19] CS activity is oftentimes used as an index of mitochondrial proliferation in muscle homogenate in the assessment of mitochondrial disorders. However, no relationship between CS activity levels and morphological evidence of mitochondrial proliferation was found in a study.[20]

METHODS

Subjects

Nineteen depressed patients (10 males; 9 females) were included in the study. The mean age of the patients at the time of muscle biopsy, performed in order to assess mitochondrial func-

tion, was 48±9 years, and their mean age at the time of ^{99m}Tc-HMPAO SPECT, performed in order to obtain a semi-quantitative measure of regional cerebral blood flow (rCBF), was 49±9 years. All patients had a chronic type of unipolar depressive disorder (>2 years) fulfilling *Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition* criteria for major depression[21] at least once. Four patients were drug-naïve for antidepressants and five patients were currently receiving medication treatment (citalopram [n=2], sertraline [n=1], fluoxetine [n=1], chlomipramine [n=1]). Substance abuse was excluded by interview in all patients.

The patients attended a tertiary psychiatric outpatient unit for clients with any type of audiological symptom. They were recruited from a group of subjects in which mean decreases of the ratios between muscle mitochondrial respiratory chain enzymes and ATP production rates were observed in comparisons with healthy controls.[13] Apart from tinnitus and/or hearing impairment, all patients also had other somatic symptoms (mild ocular/visual and muscular symptoms) that are common inmitochondrial disorders and unipolar depression.[10,13] No patient had symptoms of severe cerebral mitochondria-linked dysfunction, such as overt cognitive failure or stroke-like episodes.

The 19 patients were selected from 23 depressed patients in whom both investigations (ie, muscle biopsy and SPECT) were performed. Two of these 23 patients were omitted from the study due to an atypical muscle fibre type composition with increase of oxidative fibres was detected at light microscopy. Two other patients were excluded from the study since the number of counts were not sufficient for statistical parameteric mapping (SPM) to reach a reliable fitting. The cause may be that some ^{99m}Tc-HMPAO was injected extravasally and, thus, did not reach the bloodstream.

Ten healthy sedentary subjects, three males and seven females, with a mean age of 46±8 years served as controls for the muscle biopsy. The study was approved by the local ethics and radiation safety committees. All subjects gave informed consent.

TABLE 1.
^{99m}Tc-HMPAO SPECT Studies in Unipolar Depression*

		^{99m}Tc-HMPAO Retention	
Study (year)	***Antidepressant Treatment***	***Increased***	***Decreased***
Pagani et al (2007)[2]	30% medicated	*In atypical depressed vs non-atypical:* Bilateral frontal lobes (BA 6 + 8), parietal lobe (BAs 1–3, 5, 7, 40)	Right frontal lobe (BAs 6, 8 + 9)
Krausz et al (2007)[3]	Unmedicated for at least 3 weeks		Bilateral left > right in frontal cortex, insula, pre- and postcentral gyri, superior temporal, inferior parieto-occipital
Fountoulakis et al (2004)[4]	Unmedicated for at least 2 weeks	*In atypical depressed vs controls or other depressed groups:* Right frontal lobe	Bilateral temporal and parietal lobes, left occipital lobe, bilateral thalami, left globus pallidus in all depressed
Bonne et al (2003)[5]	Unmedicated for at least 1 week		Right parietal and occipital lobes
Gardner et al (2002)[6]	33% medicated	Right frontal lobe (BAs 8, 9, 10, 46)	
Milo et al (2001)[7]	Unmedicated	Right anterior temporal lobe	Bilateral inferior frontal lobe
Navarro et al (2001)[8]	Drug-naïve or unmedicated for 10 days		Bilateral left > right anterior frontal regions

* Comparisons between groups of depressed patients are indicated by italic font. Other comparisons were performed between depressed patients and healthy controls.

^{99m}Tc-HMPAO=^{99m}Tc-*d,l*-hexamethylpropylene amine oxime; BA=Brodmann area.

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Assessment of Mitochondrial Enzymes

Muscle biopsies were taken from the right anterior tibial muscle in all subjects. Enzyme activities were spectrophotometrically determined in isolated mitochondria. An aliquot was freeze-thawed in hypotonic medium according to the procedure by Birch-Machin and colleagues[22] The mitochondrial respiratory chain enzymes rotenone sensitive nicotinamide adenine dinucleotide-cytochrome *c* reductase (NCR [complex I + III) and succinate-cytochrome *c* reductase (SCR [complex II + III]) were determined according to Sottocasa and colleagues[23] and Cooperstein and colleagues,[24] respectively. Another aliquot was freeze-thawed in the storage medium, and treated with digitonin 2 gram L^{-1} before the analysis of the respiratory chain enzyme cytochrome *c* oxidase (COX, complex IV).[25] The activity of CS was determined according to the method by Alp and colleagues[26] after permeabilization of the mitochondria in a medium containing Triton X-100 0.05% (v/v), K_2HPO_4, 50 mmol 1^{-1} and EDTA 1 mmol 1^{-1}, pH 7.5. The enzyme activity units are expressed in spectrophotometric values.

Single Photon Emission Computed Tomography

Brain imaging using SPECT was performed using a three-headed Gamma Camera (TRIAD XLT 20, Trionix Research Laboratory, Inc., Twinsburg, Ohio) equipped with low-energy, ultrahigh-resolution collimators. The intrinsic spatial resolution of the camera was 8 mm (full width at half maximum). ^{99m}Tc-HMPAO (Ceretec, Exametazine, Amersham International Plc, Little Chalfont, United Kingdom) was injected after 30 minutes rest in a quiet, dim-lighted room. Examinations started between 45 and 60 minutes after tracer injection. The projection data were acquired for 30 seconds per projection at 90 equal angles of a complete revolution. Between 8 and 10 million total counts were acquired.

Before back-projection we pre-processed the 1-dimensional data with a Hamming smoothing filter with a cut-off frequency of 2.25 cycles/cm. Then SPECT images were reconstructed by filtered back projection algorithm using a ramp filter with a cut-off frequency of 0.6 cycles/cm. Attenuation correction was based on a 4-point ellipse.[27] No scatter correction was performed. Data were projected into a 128x128 pixel matrix resulting in an isotropic voxel size of 2.2 mm^3.

Statistical Analysis

K-means clustering was applied to create two groups of the patients according to their enzyme activities (patients with lower and higher enzymes activities). K-means clustering splits cases into a selected number of groups by maximizing the variation between groups relative to the variation within groups. It is an iterative procedure that ends when cases are successfully assigned to a specified number of non-overlapping clusters. χ^2 is used to test the distribution differences between the obtained clusters and the clinical diagnosis and type. The mean enzyme activities of the two clusters that were obtained were compared with the mean value of the healthy controls using three-groups comparisons with analysis of variance and Tukey post-hoc test. The significance level was set at $P \leq .05$.

Voxel-based Analysis

SPECT raw images were transformed into the analyze format by XMedCon package. Data were analyzed with SPM2 (Wellcome Department of Cognitive Neurology, London, UK) implemented in Matlab 6.5.1. Images of relative tracer distribution were spatially normalized into the stereotactic Montreal Neurological Institute (MNI) space to a predefined SPECT template available in SPM2 (voxel size: 2x2x2 mm), using a 16-parameter affine (non-linear) transformation. Because this template does not completely match the Talairach brain, it was necessary to correct the SPM{*t*} coordinates. This was achieved using the subroutine implemented by Brett.[28] Brodmann areas (BAs) where then identified, after importing the corrected coordinates.[29]

After normalization, images were smoothed with a Gaussian filter of 12 mm (full width at half maximum) to account for individual gyral differences and brain anatomy and to increase the signal-to-noise ratio. Images were globally normalized for signal intensity using proportional scaling to remove confounding effects due to global CBF changes, with threshold masking for grey matter of 0.8, allowing to include into the analysis only those voxels whose intensity exceeded the 80% of the maximal one. Because of the lack of any topographic a priori hypothesis, the significance of identified regions was assessed using *P* values corrected for multiple comparisons.[30,31] Significant differences between the groups were set at a threshold of $P < .05$ for cluster extent and at a threshold of $P < .005$ for voxel height. Only those

clusters containing >100 voxels were accepted as significant. This was based on the calculation of the partial volume effect resulting from the spatial resolution of the camera system.

The voxel-based analyses were performed using SPM2 with a "one scan per subject, two-sample *t*-test" design model, and significances were sought for the following contrasts: LOW minus NORMAL subtraction, and NORMAL minus LOW subtraction (for the definitions of LOW and NORMAL, see Results).

RESULTS

Creating Two Subgroups of the Patients According to the Citrate Synthase Activity

The activities of the three mitochondrial respiratory chain enzymes were significantly correlated with each other in both patients and controls (correlation coefficients of 0.66–0.78 and 0.65–0.68, respectively). The activities of CS were also significantly correlated with the activities of the individual respiratory chain enzymes in all subjects (correlation coefficients of 0.79–0.93 and 0.70–0.85, respectively) (Table 2).

The statistically most significant separation for K-means clustering was achieved using the CS activities ($F(1.17)=19.273$, $P=.000$) and two groups of patients were created according to their activities of this enzyme. Significant differences for all enzyme activities were found between the group with lower CS activities (the LOW group) and controls but not between the group with higher CS activities (the NORMAL group) and controls (Table 3).

Relationships Between the ^{99m}Tc–HMPAO Retention at SPECT and the Citrate Synthase Activity

At SPM, the LOW minus NORMAL subtraction highlighted two large significant clusters of voxels reflecting higher ^{99m}Tc –HMPAO retention in the LOW group. The clusters, bilaterally, included BAs belonging to the posterior and inferior frontal cortex, the superior and posterior temporal cortex, the somato-sensory cortex, and the associative parietal cortex (Figure and Table 4). The magnitude of the effects of SMP statistics at both cluster extent and voxel height levels and reporting (*t*) and (*z*) statistics is presented in Table 4. The NORMAL minus LOW subtraction highlighted small clusters in the bilateral orbitofrontal cortex (BAs 11 and 47) (ie, in these small regions there was decreased ^{99m}Tc –HMPAO retention in the LOW group).

DISCUSSION

The depressed patients included in this study were recruited from a specialized psychiatric unit for patients with any type of hearing impairment. Depression and hearing impairment are common manifestations of mitochondrial disorders.[10] Since mitochondria have been demonstrated to be the major subcellular fraction for the uptake of ^{99m}Tc-HMPAO in brain homogenate,[32] we speculated that differences in mitochondrial function might explain some of the discrepant results that have been reported for the retention of this tracer across studies of unipolar depression.

Voxel-based analyses (VBA) of the SPECT data demonstrated higher regional ^{99m}Tc-HMPAO retention in large portions of posterior-frontal, temporal and inferior-parietal cortex in

TABLE 2.
Correlations Between the Activities of CS and Respiratory Chain Enzymes*

	CS				NCR				SCR			
	Patients (n=19)		*Controls (n=10)*		*Patients (n=19)*		*Controls (n=10)*		*Patients (n=19)*		*Controls (n=10)*	
Enzyme	r	P	r	P	r	P	r	P	r	P	r	P
NCR	0.79	.000	0.70	.025								
SCR	0.84	.000	0.82	.004	0.66	.002	0.65	.043				
COX	0.93	.000	0.85	.002	0.74	.000	0.68	.030	0.78	.000	0.68	.031

*The relationships between the enzyme activities were evaluated with the Pearson correlation test.

CS=citrate synthase; NCR=NADH-cytochrome *c* reductase (complex I + III); SCR=succinate-cytochrome *c* reductase (complex II + III); COX=cytochrome *c* oxidase (complex IV).

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patients with low activities of the mitochondrial enzyme CS compared to patients with normal enzyme activity. There were no overt differences in mood or other depressive symptomatology between the patient groups. No differences between the patients with low and normal CS activities were found in the prefrontal cortical areas considered to be specifically involved in emotional experience and depression: BA 32 in the anterior cingulate cortex, and BA 47 in the lateral orbitofrontal cortex.[33]

We could not, for obvious reasons, measure the regional mitochondrial enzyme activities in the brain. We assumed that muscle mitochondrial function may, to some extent, reflect the mitochondrial function in brain, considering its ubiquity within tissues, even if the enzyme activities might change differently in various tissues under certain experimental conditions.[34,35]

No general pathogenetic susceptibility mechanism has been found in unipolar depression although a substantial heritable component has been reported.[9] A mouse model with multiple deletions of the mitochondrial DNA demonstrates both "mood disorder-like phenotypes" and decreased regional brain levels of the neurotransmitters serotonin and noradrenalin.[36] In muscle, altered cell histochemistry also in unmedicated patients,[13,37] decreased respiratory chain enzyme ratios and ATP production rates,[13] and an increased prevalence of small deletions of the mitochondrial DNA,[13] indicate that unipolar depression may be a systemic disorder. Multiple medically unexplained somatic symptoms, such as headache, constipation, weakness, or back pain, often conceptualized as "somatization," are reported by 50% patients with unipolar depression worldwide.[38] The audiological symptom tinnitus was present in 50% of the unmedicated depressed patients in a study.[39] A relationship between "somatization" and mitochondrial dysfunction, indicating a systemic involvement in cases of depression with somatic symptoms, has been reported.[40,41]

Decreased ATP content and protein changes suggestive of mitochondrial dysfunction have been reported in brain in unipolar depression.[11,12,14] Creatine, an agent buffering cellular ATP resources, is used in the treatment of mitochondrial disorders.[42] Beneficial effect of creatine has been reported in a preliminary study of unipolar depression refractory to antidepressant or mood-stabilizing therapies.[43] These observations, and the fact that lifetime depression was reported by >50% of the patients with mitochondrial disorders[10] suggest that mitochondrial dysfunction is among the factors that, at least, incur vulnerability to depression. In families harboring a mitochondrial DNA mutation often found in mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke-like episodes (MELAS), depressive traits were reported by 22% of asymptomatic carriers and 29% of oligosymptomatic carriers, and depressive symptoms by 42% of fully symptomatic carriers.[44]

We used the activity of CS to reflect the overall function of the mitochondrial respiratory chain and to create the two subgroups

TABLE 3.
Enzyme Activities in the Patients According to Citrate Synthase Clustering and in the Healthy Controls

	Low CS (Cluster 1)	Normal CS (Cluster 2)	Healthy Controls	ANOVA		Tukey HSD Multiple Comparisons		
	*Enzyme Activity**	*Enzyme Activity**	*Enzyme Activity**			*Low vs Normal*	*Low vs Controls*	*Normal vs Controls*
Enzyme	*8 cases*	*11 cases*	*10 cases*	*F(2,26)*	P	P	P	P
CS	1,581±431	2,533±490	2,656±732	9.14	.001	.004	.002	.877
NCR	1,316±524	2,034±932	2,398±702	4.57	.020	.125	.016	.526
SCR	710±343	1,291±310	1,405±490	7.91	.002	.010	.003	.783
COX	2,758±853	4,141±701	3,983±1,377	4.82	.017	.019	.045	.933

* The enzyme activity units are expressed in spectrophotometric values.

CS=citrate synthase; ANOVA=analysis of variance; HSD=honestly significant difference; NCR=NADH-cytochrome *c* reductase (complex I + III); SCR=succinate-cytochrome *c* reductase (complex II + III); COX=cytochrome *c* oxidase (complex IV).

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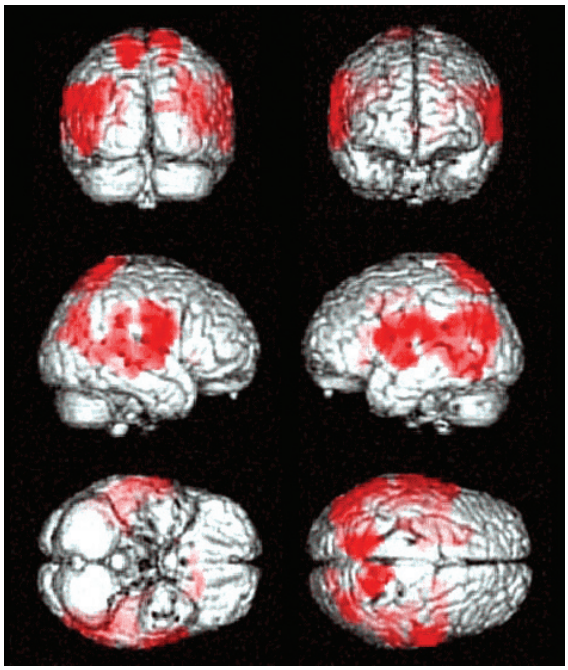
of patients. We based this choice on the finding that the best separation into two groups according to K-means clustering at using all enzyme activities was achieved with CS activity. Furthermore, CS activity was highly correlated with the activities of the respiratory chain enzymes in all subjects, adding support for the use of CS activity as a marker of the respiratory chain content and ATP production,[18,19] at least in normal or near-normal conditions. The mean CS activity in the patient group with lower activities was significantly decreased in the comparison with the healthy controls. Since all medicated patients belonged to the group with normal CS activity, antidepressant could not have been the cause of low CS activities.

Both decreased and increased regional brain distribution of the ^{99m}Tc-HMPAO retention have been reported in mitochondrial disorders.[45-50] Diffuse general hyperperfusion has been observed in MELAS patients at times when they did not suffer an acute stroke episode.[51,52]

FIGURE.
Three-dimensional rendering of voxels reflecting higher tracer distribution (red) in the depressed patients with lower muscle activity of citrate synthase compared to the depressed patients with normal citrate synthase activity



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The higher retention of ^{99m}Tc-HMPAO in patients with low CS activity may reflect local hemodynamic changes. Intracellular metabolic dysfunction is followed by increased rCBF in severe mitochondrial pathology, such as the MELAS syndrome, thus producing the so-called "luxury perfusion". This phenomenon has been suggested to represent adaptation to the altered mitochondrial function, leading to impaired intracellular oxygen utilization and metabolism. It was speculated that increased CBF might be a compensation for increased wash-out of lactate produced by increased anaerobic metabolism,[52] or might have been related to decreased pH caused by local increase of lactic acid.[48] Quantitative CBF measurements with xenon^{133} demonstrated diffuse hyperperfusion in a young man affected by MELAS years prior to undergoing a large posterior "metabolic" stroke.[51] These findings were confirmed by a PET study of a MELAS patient in which decreased cerebral metabolic rate of dioxygen was found along with increased CBF and cerebral metabolic rate of glucose.[52]

Unevenly distributed activities in the brain have been reported for mitochondrial respiratory chain enzymes.[53] In MELAS specifically, mitochondrial dysfunction is widespread but, in the brain, it predominates in the posterior areas.[54] Different cellular thresholds to metabolic dysfunction in the various brain areas, according to local dependence on oxidative metabolism, have been suggested to be one of the mechanisms leading to the regional, rather than general, expression of neuropathologic lesions in mitochondrial disorders.[55-58] This would result in a subpopulation(s) to be selectively more affected by a generalized impairment and is consistent with the reported quantitative CBF measurements in mitochondrial disorder in which increased CBF was present in all brain, with regional pronouncements.[51] Our results suggest that scattered brain regions in the posterior frontal, temporal, and parietal cortices are the most sensitive to mildly impaired mitochondrial function.

The conversion of the lipophilic tracer ^{99m}Tc-HMPAO into the hydrophilic form that is retained in the cell has been related to the cellular GSH content.[15,16] GSH deficiency has been reported in the most severe cases of mitochondrial disorders.[59] On the other hand, increased GSH levels, considered to reflect GSH upregulation at increased oxidative stress secondary to reduced

respiratory chain enzyme activity, were found in the early stages of mitochondrial disorders.[17] The higher tracer retention that was observed in scattered brain regions in the patients with low CS activity may partially reflect such early increased GSH content.

A limitation of the study is that the approach used did not allow to discriminate to which portion ^{99m}Tc-HMPAO retention was related to either CBF or intracellular enzymatic changes. Other limitations are the relatively small number of subjects, and that the muscle biopsies and SPECT investigations were not performed simultaneously. Our subjects were recruited from two separately ethically approved studies since it would not have been possible to obtain an ethical permission for two invasive trials, and most likely to recruit enough subjects, for such a study. Since the physical activity levels of the depressed subjects did not differ between the investigations 1 year apart as determined by interview, this should not have affected the results. We did not compare the SPECT imaging results to healthy SPECT controls since the inclusion of two different control groups in one study is considered inappropriate. Comparisons between the SPECT results of our patients and controls are also beyond the purpose of this study.

TABLE 4.
SPM Statistics Relative to the Subtraction Image Resulting From LOW Minus NORMAL Groups of Citrate Synthase Activity

Cluster Level		Voxel Level				Talairach Coordinates			Anatomical and Functional Location
P(cor)	Number of Voxels	P(FDR-cor)	t	z score of Maximum	P(unc)	x	y	z	
.000	1,746	.066	6.16	4.41	.000	–42	–69	23	L Middle temporal gyrus BA 39
		.066	5.04	3.89	.000	–48	–47	2	L Middle temporal gyrus BA 21
		.066	4.82	3.78	.000	–48	–31	15	L Superior temporal gyrus BA 29
		.066	4.44	3.57	.000	–42	4	25	L Inferior frontal gyrus BA 9
		.068	4.21	3.44	.000	–59	–45	24	L Supramarginal gyrus BA 40
		.068	4.17	3.42	.000	–24	–23	15	L Claustrum
		.075	4.01	3.32	.000	–56	–54	28	L Supramarginal gyrus BA 40
		.075	4.00	3.31	.000	–53	–60	31	L Superior temporal gyrus BA 39
		.076	3.94	3.27	.001	–53	7	16	L Inferior frontal gyrus BA 44
		.077	3.91	3.26	.001	–65	–11	23	L Postcentral gyrus BA 3
		.080	3.79	3.18	.001	–59	–25	21	L Postcentral gyrus BA 40
		.080	3.77	3.17	.001	–56	–6	–2	L Superior temporal gyrus BA 22
		.080	3.74	3.15	.001	–62	9	13	L Precentral gyrus BA 44
		.082	3.67	3.11	.001	–59	–8	17	L Postcentral gyrus BA 43
		.091	3.43	2.95	.002	–50	–19	31	L Postcentral gyrus BA 2
		.094	3.33	2.88	.002	–39	–70	1	L Middle occipital gyrus BA 37
.000	1,334	.066	5.67	4.19	.000	62	–8	20	R Postcentral gyrus BA 43
		.066	5.09	3.91	.000	42	–66	23	R Middle temporal gyrus BA 39
		.066	4.65	3.68	.000	56	–34	13	R Superior temporal gyrus BA 42
		.068	4.27	3.47	.000	45	–52	8	R Superior temporal gyrus BA 39
		.076	3.94	3.27	.001	62	–26	–4	R Middle temporal gyrus BA 21
		.077	3.88	3.24	.001	30	–68	39	R Precuneus BA 19
		.080	3.79	3.18	.001	42	–39	32	R Supramarginal gyrus BA 40
		.081	3.72	3.14	.001	48	4	22	R Inferior frontal gyrus BA 9
		.083	3.61	3.06	.001	33	4	30	R Precentral gyrus BA 6
		.072	3.18	2.78	.003	39	–22	31	R Postcentral gyrus BA 2
		.074	3.12	2.73	.003	62	–30	32	R Inferior parietal lobule BA 40
		.076	3.07	2.70	.003	12	–80	23	R Cuneus BA 18
		.079	2.97	2.63	.004	24	–78	23	R Cuneus BA 18
		.082	2.91	2.59	.005	27	–32	2	R Thalamus

SPM=statistical parametric mapping; cor=corrected; FDR-cor=false discovery rate-corrected; unc=uncorrected; BA=Brodmann area; L=left; R=right.

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CONCLUSION

In conclusion, in support of our hypothesis we found higher retention of the tracer ^{99m}Tc-HMPAO in large portions of some brain regions in depressed patients with overall decreased, as opposed to overall normal, mitochondrial enzyme activities. Higher ^{99m}Tc-HMPAO retention might reflect a local perfusion increase and higher intracellular levels of GSH, both due to the regional biochemical changes following mitochondrial dysfunction. Studying the involvement of mitochondrial energy production is a cumbersome endeavor since almost a thousand proteins are involved. An implicit suggestion arising from our study is the use of tracers designed to assess mitochondrial functions in order to estimate the prevalence, the clinical features, and the neurobiological substrates of depression associated with mitochondrial dysfunction. ***CNS***

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