Brain Bioenergetics and Response to Triiodothyronine Augmentation in Major Depressive Disorder

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Background: Low cerebral bioenergetic metabolism has been reported in subjects with major depressive disorder (MDD). Thyroid hormones have been shown to increase brain bioenergetic metabolism. We assessed whether changes in brain bioenergetics measured with phosphorus magnetic resonance spectroscopy (^{31}P MRS) correlate with treatment outcome during augmentation treatment with triiodothyronine (T3) in MDD.

Methods: Nineteen subjects meeting DSM-IV criteria for MDD who had previously failed to respond to selective serotonin reuptake inhibitor (SSRI) antidepressant drugs received open label and prospective augmentation treatment with T3 for 4 weeks. We obtained ^{31}P MRS spectra before and after treatment from all MDD subjects and baseline ^{31}P MRS from nine normal control subjects matched for age and gender.

Results: At baseline, depressed subjects had lower intracellular Mg^{2+} compared with control subjects. Seven MDD subjects (38.9%) were treatment responders ($\geq$ 50% improvement). Total nucleoside triphosphate (NTP), which primarily represents adenosine triphosphate (ATP), increased significantly in MDD subjects responding to T3 augmentation compared with treatment nonresponders. Phosphocreatine, which has a buffer role for ATP, decreased in treatment responders compared with nonresponders.

Conclusions: The antidepressant effect of thyroid hormone (T3) augmentation of SSRIs is correlated with significant changes in the brain bioenergetic metabolism. This seems to be a re-normalization of brain bioenergetics in treatment responders. Further studies will determine whether these findings can be generalized to other antidepressant treatments.

Key Words: Bioenergetic metabolism, magnetic resonance spectroscopy, major depressive disorder, treatment response, triiodothyronine, T3

Multiple studies have reported regional and global hypometabolism in major depressive disorder (MDD), which could be related to the neurobiology of mood disorders. Positron emission tomography (PET) studies have shown abnormalities in glucose use rates and blood flow in several brain regions of subjects with major depression (1,2). Moreover, metabolic abnormalities in the anterior cingulate and the amygdala/hippocampus complex seem to improve after antidepressant treatment (3,4). Proton (1H) magnetic resonance spectroscopy (MRS) studies have shown abnormalities of the energy-intensive cellular membrane phospholipid metabolism, as measured by altered choline/creatine ratios, in the orbitofrontal cortex of depressed subjects (5). The cytosolic choline-containing compounds (mainly phosphocholine and glycerophosphocholine) contributing to the 1H MRS choline peak play an important role in brain cell membrane phospholipid synthesis, which requires a large fraction of brain cells' available adenosine triphosphate (ATP) (6). Thus, these observations of altered brain 1H MRS choline levels in depressed patients are consistent with altered brain energy metabolism.

To date, only a limited number of studies have addressed changes in brain energy metabolism as measured with phosphorus (^{31}P) MRS in MDD subjects. This literature, which we have reviewed previously (7), describes several abnormalities of bioenergetic metabolism, primarily decreased baseline levels of β-nucleoside triphosphate (β-NTP) and total NTP, in the basal ganglia and the frontal lobes of MDD subjects compared with normal control subjects (8–10). However, there is currently only one study (11), with n = 2 subjects, that used repeated ^{31}P MRS to assess changes in bioenergetic metabolism in relation with the outcome of antidepressant treatment. Therefore we do not know whether previously reported brain bioenergetic abnormalities in MDD represent a biological trait of subjects at risk for MDD or whether they are dependent on the state and severity of depression.

Thyroid hormones, especially triiodothyronine (T3) (12), have been shown effective as an augmentation strategy in treatment-resistant depression (TRD). Most of the published data support their role for augmentation of tricyclic antidepressant drugs (13). More recently some studies have described moderate efficacy of thyroid hormones as adjuvants to selective serotonin reuptake inhibitors (SSRI) in TRD (14–17).

Because thyroid hormones were shown to increase bioenergetic metabolism in skeletal muscle (18) and in the brain (19) of hypothyroid subjects, we hypothesized that the antidepressant role of thyroid hormones might be related to their activity on the brain bioenergetic metabolism (7). Thus we hypothesized that MDD subjects responding to T3 augmentation will present significant increases of brain bioenergetic metabolism, as reflected by brain NTP and phosphocreatine (PCr) levels, compared with treatment nonresponders (7). The following study was designed to test this hypothesis.

Methods and Materials

Subjects

Twenty depressed subjects were recruited through advertisements and clinical referrals for a treatment study at Massachusetts

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General Hospital between 2001 and 2003 (16). Nineteen of the 20 subjects were eligible and agreed to undergo ^{31}P MRS scans before and after treatment. Institutional Review Board (IRB)-approved written informed consent was obtained from all study participants.

All depressed subjects were between ages of 18 and 65 and met criteria for MDD diagnosed by physician-administered Structured Clinical Interview for DSM-IV Axis I Disorders (SCID) (20); all had a score of ≥ 16 on the 17-item Hamilton Rating Scale for Depression (HAMD-17) (21) at the screen visit. All MDD subjects had previously shown minimal or no response to treatment with an SSRI taken for ≥ 8 weeks, with ≥ 4 weeks at a stable dose (fluoxetine ≥ 40 mg/day, sertraline ≥ 100 mg/day, paroxetine ≥ 40 mg/day, citalopram ≥ 40 mg/day, escitalopram ≥ 20mg/day).

The exclusion criteria included inadequate contraception, pregnancy, lactation, serious suicidal risk, serious or unstable medical illness, medical disorders where T3 treatment was contraindicated, abnormal baseline thyroid-stimulating hormone (TSH) levels, substance use disorders (including alcohol) active within the last year, any psychotic disorder, bipolar disorder, history of multiple adverse drug reactions or hypersensitivity to T3, and any contraindication to ^{31}P MRS (metallic foreign bodies, claustrophobia, morbid obesity).

We also enrolled nine normal control subjects matched for age and gender who were screened with SCID (20) to exclude any Axis I psychiatric disorder. All normal control subjects were not medicated and had no medical or neurological history.

Treatment

After the initial evaluation, all MDD subjects enrolled into a 4-week open treatment with triiodothyronine (T3) 50 μg daily added to their existing SSRI, after first undergoing a 1-week evaluation phase. The HAMD-17 scale was administered every 2 weeks. The MDD subjects had TSH, T3, T4, and free-T4 levels measured before initiation of treatment and at 4-weeks follow-up with a solid-phase radioimmunoassay (Massachusetts General Hospital Laboratories). Detailed results of the open trial of T3 augmentation of SSRIs have been reported previously (16).

Magnetic Resonance Spectroscopy

All subjects underwent brain ^{31}P MRS in the 4-T Varian Unity/Inova (Varian, Palo Alto, California) magnetic resonance scanner at the Brain Imaging Center at McLean Hospital. All scans were acquired with a dual tuned, dual quadrature detection, open-face proton-phosphorus TEM whole-head coil (MR Instruments, St. Louis Park, Minnesota) operating at nominal frequencies of 170.3 MHz for 1H and 68.9 MHz for ^{31}P. We obtained two scans for MDD subjects (at baseline and at the end of the study) and one scan for normal control subjects. The ^{31}P MRS visit included subject positioning, frequency and field homogeneity shimming adjustments over the whole head, acquisition of a series of pilot images, positioning of the slice for ^{31}P MRS, shimming on the slice, and acquisition of ^{31}P MR spectra from the brain. The head was positioned reproducibly relative to the center of the scanner bore and radiofrequency (RF) coil over sessions for each subject with the patient table laser beam. Pilot proton images were acquired with multi-slice rapid gradient-echo proton magnetic resonance imaging in the sagittal, coronal, and axial planes. Sagittal pilot images were used to reproducibly position the 20-mm-thick axial brain slice used for acquisition of the ^{31}P MRS data from session to session. Axial proton images of the ^{31}P MRS brain slice acquired as one 20-mm-thick slice and as five adjacent 4-mm-thick slices were used to estimate the contribution of head skin and muscle tissue to the ^{31}P MRS acquisition volume. The 20-mm-thick axial brain slice was prescribed through frontal and parietal areas, above the corpus callosum as shown in Figure 1. The slice was positioned mid-sagittally so as to center the slice in the anterior cingulate cortex. The gradient recalled echo of the ^{31}P MRS free induction decay signal was acquired from the slice with a standard slice selective sequence. The sequence was implemented by applying a 90° flip-angle 5000 μs-duration frequency-selective 5-lobe sinc-shaped RF pulse simultaneously with a gradient in the slice selection direction, followed by a 2.5-msec phase refocusing gradient and acquisition of the signal. The following acquisition parameters were applied: number of averaged transients NT = 128, number of acquired complex points NP = 2048, spectral width SW = 4000 Hz, repetition time TR = 2000 msec, number of dummy transients DS = 4. Application of the refocusing gradient resulted in a delay between RF pulse and acquisition of 2.554 msec. The total scan time was 4 min and 57 sec. The 2-sec repetition time resulted in a substantial attenuation of the phospholipid metabolism peaks (phosphomonoesters [PME] and phosphodiesters

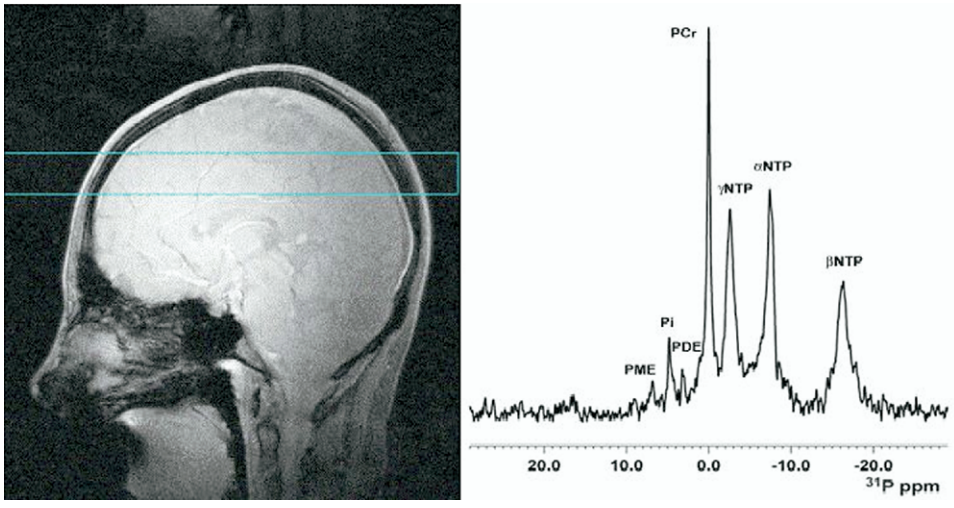

Figure 1. The position of the phosphorus magnetic resonance spectroscopy (^{31}P MRS) acquisition slice (left) and a sample ^{31}P MRS spectrum recorded from a healthy volunteer (right). The position of the slice for acquisition of ^{31}P MRS data is indicated by lines overlaid on the mid-sagittal proton image. PME, phosphomonoesters; Pi, inorganic phosphate; PDE, phosphodiesters; PCr, phosphocreatine; NTP, nucleoside triphosphate.

[PDE], see Figure 1 and following text) relative to the NTP peaks, owing to differential longitudinal relaxation (T1) saturation effects (at 4T, the PME and PDE T1s are approximately 2.7 and approximately 3.9 sec, respectively, whereas the T1 of ATP is approximately 1.0 sec (22). This relative signal attenuation due to differential saturation was consistent for all scans.

Spectral resonances were assessed with fully automated, time domain fitting algorithms. Analysis of ^{31}P MRS in vivo data was performed with a time domain spectral fitting routine based on a nonlinear, Marquardt-Levenburg algorithm in combination with prior spectral knowledge (22). The individual estimated areas of the peaks from phosphomonoesters (PME), inorganic phosphate (Pi), phosphodiesters (PDE), PCr, and nucleoside triphosphate (γ, α, and β NTP) were normalized by the total phosphorus signal, yielding the percentage of total phosphorus signal intensity contributed by the metabolite peak. The intracellular pH was determined from the chemical shift of Pi relative to PCr (23). The intracellular concentration of free magnesium (Mg^{2+}) was determined from the chemical shift of β-NTP relative to PCr with a semi-empirical equation, taking into account the brain cytosolic pH and the intracellular ionic strength (24). This method of assessing cytosolic free Mg^{2+} concentration is considered to be more accurate than methods relying on the measurement of the chemical shift difference between α- and β-NTP, mainly because of the unresolved resonances of α adenosine diphosphate (ADP), NAD, and NADH (NAD and NADH are the two forms of nicotinamide adenine dinucleotide) underlying upfield the α-ATP peak (24). The researcher (NRB) performing all metabolite evaluations was blinded to the clinical status of all study subjects.

To measure the reliability of our methods we acquired ^{31}P MRS data on four healthy human volunteers at 4T, each scanned at two separate times (1 week apart), with the same sequence and brain slab positioning as described previously. We measured ^{31}P MRS metabolite values and the coefficients-of-variance for high-energy phosphate metabolites. For the four subjects the average coefficients of variation between visits were PCr = 4% (range =1%–10%), total NTP = 2% (range = .3%–3%), β NTP = 2% (range = 1%–3%), pH = .07 % (range = .01%–.23%), Mg^{2+} = 5.6% (range = .6%–8.5%).

Statistical Analysis

Given the small sample size, group differences in demographic and clinical variables were analyzed with nonparametric tests (Wilcoxon signed rank test for paired data, Wilcoxon rank sum test for unpaired data) and χ^2 tests. We used the nonparametric Wilcoxon rank sum (Mann-Whitney *U*) test to compare baseline levels of ^{31}P MRS metabolites between MDD subjects and normal control subjects. Clinical outcome was defined as response to treatment (HAMD-17 reduction ≥ 50% during treatment). We also used the Wilcoxon rank sum (Mann-Whitney *U*) test to compare changes from baseline to end of study in ^{31}P MRS metabolites between treatment responders and nonresponders. We used the same test to compare baseline levels of ^{31}P MRS metabolites between treatment responders and nonresponders. The three main comparisons (1. comparisons of MDD vs. control subjects; 2. comparisons of baseline ^{31}P MRS metabolites in responders vs. nonresponders; and 3. comparisons of changes in MRS metabolites in responders vs. nonresponders) were each tested at an overall α level of .05. Because each of the three comparisons included five metabolites (β-NTP, total NTP, PCr, pH, and Mg^{2+}), the *p* values obtained for individual metabolites were multiplied by 5 to perform Bonferroni corrections for multiple comparisons. In secondary analyses we used linear regression to assess the relationship between clinical depression improvement (% change HAMD-17) and changes in ^{31}P MRS metabolites, controlling for age and gender (and we also performed the Bonferroni correction described herein). All analyses were performed with Stata 9.0 for Windows (Stata Corporation, College Station, Texas). Statistical significance was defined at the $p < .05$ level, two-tailed.

Results

The demographic and clinical characteristics of our subjects are presented in Table 1. After 4 weeks of treatment, the mean severity of depression dropped from HAMD-17 = 20.3 ± 3.6 to HAMD-17 = 13.3 ± 6.6. Seven subjects (36.8%) were treatment responders (HAMD-17 reduction ≥ 50%) and six subjects (31.6%) achieved clinical remission (final HAMD-17 ≤ 7). The results of the open T3 augmentation trial have been reported in detail previously (16). Spectral data on one baseline scan (from a depressed subject, treatment responder) and two post-treatment scans (treatment nonresponders) were of poor quality and could not be used, yielding complete pre- and post-treatment data for 16 subjects (6 treatment responders, 10 nonresponders).

The baseline intracellular free Mg^{2+} was significantly lower in depressed subjects compared with normal control subjects [$z(25) = -2.78$, corrected $p = .03$]. There were no significant differences in baseline levels of β NTP, total NTP, PCr, and pH between MDD and control subjects (Table 2).

Baseline PCr levels were numerically higher in MDD subjects who responded to T3 augmentation compared with nonresponders, but the difference did not reach statistical significance after correcting for multiple comparisons [$z(14) = -2.435$, corrected $p = .064$]. Baseline PCr levels predicted treatment

Table 1. Clinical Characteristics of MDD Subjects and Normal Control Subjects

	MDD Subjects ($n = 19$)	Normal Control Subjects ($n = 9$)	Test Statistic	*p*
Age	43.6 ± 10.0	40.0 ± 10.1	$z(26) = -1.06$	.29
Female Gender	11 (57.9%)	5 (55.5%)	$\chi^2(1) = .44$	.51
SSRI Dose (equivalent mg fluoxetine)	53.7 ± 29.9			
Baseline HAMD-17 score	20.3 ± 3.6	.4 ± .7	$z(26) = -4.24$	$p < .0001$[a]
Baseline TSH (mIU/L)	1.75 ± .71	1.97 ± 1.22	$z(26) = .47$	.64
Final TSH (mIU/L)	.10 ± .24			

MDD, major depressive disorder; SSRI, selective serotonin reuptake inhibitor; HAMD-17, 17-item Hamilton Rating Scale for Depression (treatment response = HAMD-17 improvement > 50%); TSH, thyroid-stimulating hormone.
[a] $p < .05$.

Table 2. Baseline ^{31}P MRS Metabolite Levels in MDD Subjects and Normal Control Subjects

	MDD Subjects (n = 18)	Normal Control Subjects (n = 9)	Test Statistic	p
Baseline β NTP	16.13 ± 3.40	18.03 ± 2.60	$z(25) = 1.65$	corrected, .50
Baseline Total NTP	51.85 ± 5.72	54.75 ± 5.66	$z(25) = 1.54$	corrected, .60
Baseline PCr	24.48 ± 2.85	22.40 ± 1.76	$z(25) = -2.16$	corrected, .15
Baseline pH	7.04 ± .03	7.02 ± .02	$z(25) = -.51$	corrected, ns
Baseline Mg^{2+} (μmol/L)	152.2 ± 22.7	193.8 ± 32.8	$z(25) = -2.78$	corrected, .03[a]

^{31}P MRS, phosphorus magnetic resonance spectroscopy; MDD, major depressive disorder; NTP, nucleoside-triphosphate; PCr, phosphocreatine.

[a] $p < .05$ after Bonferroni correction for multiple comparisons.

response with 79% accuracy (83% sensitivity, 75% specificity) and .88 Area Under the Receiver Operating Curve (AUC) (Figure 2). There were no significant differences in baseline β NTP, total NTP, pH, and cytosolic Mg^{2+} between responders and nonresponders to T3 augmentation ($p > .05$).

Compared with depressed subjects not responding to thyroid hormone augmentation treatment, treatment responders experienced significant increase in total NTP and a compensatory decrease in PCr, which has a buffer role for ATP (Table 3 and Figure 3). In linear regression analyses the changes in total NTP and PCr during treatment were significantly associated with depression improvement (% change HAMD-17), when adjusting for age and gender (Supplement 1). The associations remained significant when additionally adjusting for intracellular pH and Mg^{2+}.

Discussion

To our knowledge, this is the first study to report differential changes in the brain bioenergetic metabolism between treatment responders and nonresponders in MDD. This is also the first study to suggest that baseline PCr levels could be a predictor of treatment outcome in depression.

Brain levels of total NTP increased from baseline to end of treatment in treatment responders but not in nonresponders. In the brain NTP levels primarily reflect ATP, which is present at a much higher concentration than other NTP (25,26). Our data suggest that previously reported abnormalities of brain energy metabolism (low baseline ATP levels) in MDD tend to re-normalize only in subjects responding to antidepressant treatment with T3 augmentation but not in treatment nonresponders. Larger studies with standard antidepressant drugs will be needed to determine whether changes in bioenergetic metabolism are a general brain mechanism involved in the recovery from depression or if this is a more specific mechanism related only to the antidepressant activity of thyroid hormones.

We also found that brain levels of PCr decreased from baseline to end of treatment in treatment responders but not in nonresponders. In treatment responders ATP levels increased during treatment, whereas PCr levels decreased. These compensatory changes in brain PCr levels might be related to the buffer role of PCr in relation to ATP. During physiological stimulation the brain concentration of ATP is maintained constant at the expense of PCr. Phosphocreatine transfers high-energy phosphate groups to ADP, re-forming ATP in a reaction mediated by creatine kinase (27). However, compensatory changes in PCr related to changes in ATP have also been reported in healthy volunteers, before and after treatment with creatine (28) or with S-adenosine methionine (SAMe) (29). Therefore, these compensatory changes in PCr in rapport to changes in ATP might occur in a variety of situations leading to overall changes in the steady-state equilibrium of the brain bioenergetic metabolism. Of note, a study of two geriatric depressed subjects had previously suggested that PCr levels decrease with improvement of depression (11), but the small sample size ($n = 2$) makes those results less reliable.

Iotti *et al.* (30) have shown that in the brain the equilibrium between intracellular ATP and ADP is dependent on PCr, pH, and Mg^{2+}. We have therefore used intracellular pH and Mg^{2+} as covariates in our linear regression analyses; their presence did not change the significant relations between improvement of

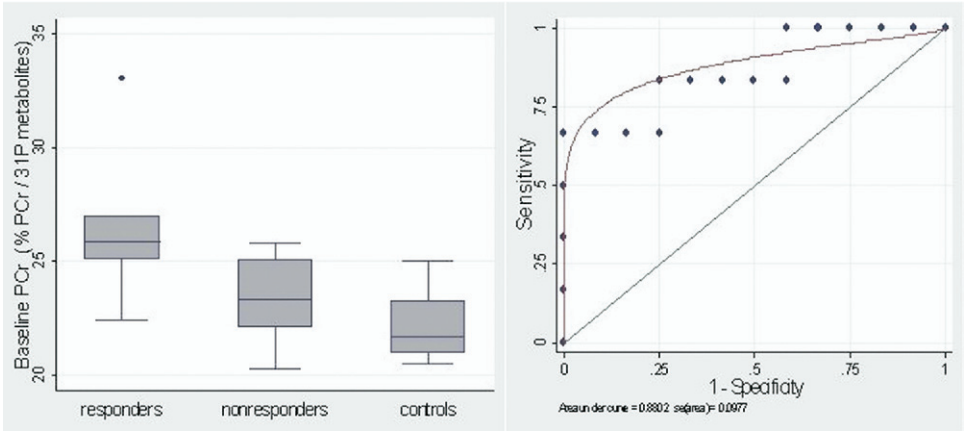

Figure 2. Baseline phosphocreatine (PCr) levels in two groups of major depressive disorder subjects (treatment responders and nonresponders) and in normal control subjects. On the right, baseline PCr seems to be a good predictor of treatment response (83% sensitivity, 75% specificity, .88 area under the receiver-operator curve).

Table 3. Change (%) in High-Energy Metabolite Levels During the 4-Week Treatment With T3

	Treatment Responders	Treatment Nonresponders	Wilcoxon Rank Sum Test Statistic	*p* (adjusted for multiple comparisons)	Association With Clinical Improvement (% change HAMD-17)
βNTP	4.66 ± 5.40	−.99 ± 2.10	$z(14) = -2.50$	corrected, .065	coef = .05 $t(3, 12) = 2.40$, corrected $p = .17$
Total NTP	8.70 ± 5.74	−2.23 ± 4.42	$z(14) = -3.15$	corrected, .01[a]	coef = .03 $t(3, 12) = 3.19$, corrected $p = .04$[a]
PCr	−5.07 ± 3.93	−.24 ± 1.91	$z(14) = 2.71$	corrected, .03[a]	coef = −.70 $t(3, 12) = -3.35$, corrected $p = .03$[a]
pH	−.01 ± .06	.02 ± .06	$z(14) = -1.06$	corrected, ns	coef = −1.50 $t(3, 12) = -1.03$, corrected p = ns
Mg^{2+} (μmol/L)	16.2 ± 22.9	20.4 ± 53.1	$z(14) = -.24$	corrected, ns	coef = −.04 $t(3, 12) = -1.43$, corrected p = ns

Comparison between responders and nonresponders (nonparametric Wilcoxon rank sum test) and association between changes in metabolite levels and clinical improvement (linear regression, adjusted for age and gender). T3, triiodothyronine; HAMD-17, 17-item Hamilton Rating Scale for Depression; NTP, nucleoside-triphosphate; PCr, phosphocreatine.

[a] $p < .05$ after Bonferroni correction for multiple comparisons.

depression and changes in ATP and PCr levels during the study. To the extent that our findings can be generalized to antidepressant treatments other than thyroid hormones, it would justify further research on other compounds that modify brain energy metabolism and also have promising antidepressant activity, such as SAMe (31). Changes in the brain bioenergetic metabolism might also become a useful pharmacological target in the search for future antidepressant drugs.

Ours is also the first study to suggest that baseline PCr levels could be a predictor of treatment outcome in depression. In our dataset PCr predicted response to T3 augmentation with good specificity and sensitivity and a .88 AUC. Baseline PCr was significantly higher in MDD subjects who responded to antidepressant treatment compared with nonresponders. If our current results, on the basis of a small sample and treatment with a nonstandard antidepressant (T3), were replicated in a larger group with a first line antidepressant, the performance of baseline PCr as a predictor of antidepressant response would be superior to other clinical and biological predictors currently proposed (32).

Renshaw *et al.* (10) had previously suggested that baseline β NTP levels could differentiate between future treatment responders and nonresponders. In our study treatment responders had, similar with their results (10), lower baseline β NTP (15.07 ± 2.13) versus treatment nonresponders (16.66 ± .62), but those differences were not statistically significant. Our results suggesting PCr as a possible biomarker of treatment response are consistent with the results of Renshaw *et al.* (10), because both studies unveil a set of bioenergetic abnormalities in depression that could be related to mitochondrial dysfunction. These abnormalities might characterize treatment responders from nonresponders. Our post treatment data suggest those abnormalities tend to be corrected by successful antidepressant treatment with T3.

We found no significant differences in baseline NTP and baseline PCr levels between MDD and control subjects after adjusting for multiple comparisons. However, the magnitude of the differences in baseline metabolites observed here is consistent with previous studies. We found total NTP was decreased by 5.7% in MDD subjects versus control subjects, whereas in previous studies total NTP was decreased by 7.6% (9) and by 6.6% (8) in MDD patients compared with control subjects. In our group baseline PCr was 9.3% higher in MDD versus control subjects, whereas previous reports found PCr to be 8.5% higher (9) or 4.1% higher (8) in MDD versus control subjects.

We found that baseline intracellular Mg^{2+} levels were lower in the brains of depressed subjects than in healthy volunteers. This finding raises the question of a possible role of impaired magnesium homeostasis in MDD. Magnesium is a coenzyme in numerous enzymatic reactions and a co-factor for ATP; energy is released from the ionic species Mg-ATP by the adenylate kinase reaction, and free intracellular Mg^{2+} levels modulate the ATP buffering creatine kinase reaction (33). Several studies reported that serum Mg levels were altered in MDD as well as in depressed bipolar patients (34–36). Previous reports have associated the antidepressant effect of lithium in MDD with high baseline serum Ca/Mg ratio (37) and with fluctuations in plasma calcium and

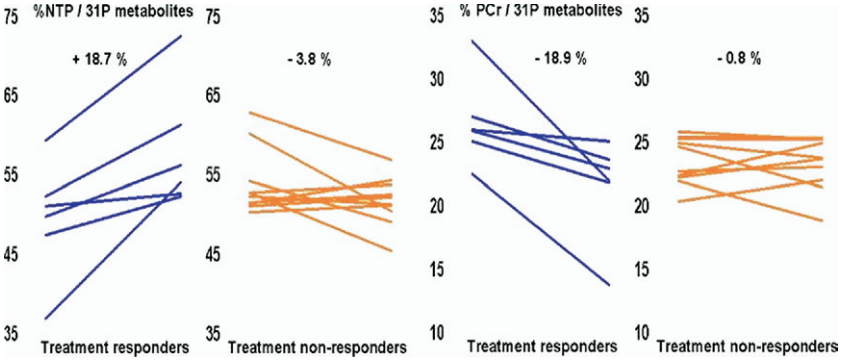

Figure 3. Changes in total nucleoside triphosphate (NTP) levels (left) and phosphocreatine (PCr) levels (right) during treatment in two groups of major depressive disorder subjects (treatment responders and nonresponders).

magnesium during treatment (38). In our study neither baseline brain Mg^{2+} nor was the change in brain Mg^{2+} levels during thyroid augmentation significantly different between responders and nonresponders, whereas higher baseline PCr levels normalized in treatment responders. This suggests that the underlying mechanism related to antidepressant response is more likely associated with the regulation of PCr by creatine kinase.

The lower baseline intracellular Mg^{2+} concentration in the brains of MDD subjects compared with control subjects could be explained by impaired oxidative phosphorylation related to mitochondrial dysfunction. Low brain intracellular Mg^{2+} was also found in patients with mitochondrial cytopathies (39) and migraine and cluster headache (40). In mitochondrial cytopathies therapeutic interventions improving respiratory chain efficiency resulted in normalization of Mg^{2+}, thus suggesting that low Mg resulted from failure of the respiratory chain (39). Lodi *et al.* (40) concluded as well that decreased Mg^{2+} concentrations in migraine headaches were secondary to the bioenergetics deficit. This could also be the case with MDD subjects in our study.

The limitations of our study include a small sample size and a relatively short duration of the treatment study (4 weeks). Although we followed previous studies in the literature (12,41,42), which reported efficacy of T3 augmentation after 4 weeks or less, it is possible that a longer study might have led to different results. It is also not clear whether the time required for changes in bioenergetic metabolism is the same as the time required for the clinical antidepressant response. Because all subjects were taking SSRI antidepressant drugs at baseline, we cannot assess the impact of such antidepressant drugs on baseline MRS metabolite measures. However, our results are consistent with studies of unmedicated MDD subjects (8–10). In our study, the ^{31}P MRS data were acquired from a 20-mm-thick axial slice through the head. Therefore we could not measure changes in NTP and PCr levels in specific brain areas involved in mood regulation. Moreover, our measurements of brain NTP and PCr likely contained contributions of phosphorus signal from metabolites in the skin and muscle tissue (no ^{31}P MRS signal is expected from bone, owing to extreme line broadening in the solid state). We segmented and measured tissue volumes in the proton images acquired with our slice prescription. The total volume of skin, muscle, and bone tissue represents approximately 15% of the acquisition volume. We estimate the muscle tissue alone represents approximately 5% of the acquisition volume, which is consistent with previous studies (43), which used a similar slice position and found a 5% contribution of muscle to the tissue volume in the slice. Given that the concentration of ATP and PCr is higher in muscle tissue than in brain (44,45), we estimate that 10%–14% of the NTP signal and 15%–30% of the PCr signal recorded in our brain slab might originate in scalp muscle. Thyroid hormones have been shown to increase bioenergetic metabolism in skeletal muscle (18) as well as in the brain (19). It is therefore possible that thyroid hormones could engender bioenergetic metabolic changes in brain and muscle tissue alike and that such changes (occurring throughout the body) would be related to clinical response to thyroid hormones in depression. In contrast, our interpretation of these data is that changes in ^{31}P MRS metabolites are related primarily to changes in brain metabolism, changes that occur selectively in treatment responders. Our interpretation is consistent with our more recent results (Iosifescu *et al.*, unpublished, presented at SOBP Annual Meeting 2007) where ^{31}P MRS metabolite changes were noted in brain-only voxels of responders (but not in nonresponders) after treatment with standard antidepressant drugs. But the data presented here do not separate sufficiently the muscle contribution to the ^{31}P MRS metabolite changes. Additional studies will be needed to completely elucidate the brain (and the brain region) contribution to these changes in ^{31}P MRS metabolites.

Despite these limitations, our results suggest that depressed subjects have abnormal brain bioenergetic metabolism and that the antidepressant effect of thyroid hormone (T3) augmentation of SSRIs is correlated with significant changes in brain bioenergetics, primarily with increases in brain ATP levels and with compensatory decreases in brain PCr. This might be a more general brain mechanism involved in the recovery from depressive episodes. Further studies are needed to determine whether these findings can be generalized to other antidepressant treatments.

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Supplementary material cited in this article is available online.

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