

**FEATURE REVIEW**

# Mitochondrial dysfunction in bipolar disorder: evidence from magnetic resonance spectroscopy research

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**Magnetic resonance spectroscopy (MRS) affords a noninvasive window on *in vivo* brain chemistry and, as such, provides a unique opportunity to gain insight into the biochemical pathology of bipolar disorder. Studies utilizing proton ( $^1\text{H}$ ) MRS have identified changes in cerebral concentrations of *N*-acetyl aspartate, glutamate/glutamine, choline-containing compounds, myo-inositol, and lactate in bipolar subjects compared to normal controls, while studies using phosphorus ( $^{31}\text{P}$ ) MRS have examined additional alterations in levels of phosphocreatine, phosphomonoesters, and intracellular pH. We hypothesize that the majority of MRS findings in bipolar subjects can be fit into a more cohesive bioenergetic and neurochemical model of bipolar illness that is both novel and yet in concordance with findings from complementary methodological approaches. In this review, we propose a hypothesis of mitochondrial dysfunction in bipolar disorder that involves impaired oxidative phosphorylation, a resultant shift toward glycolytic energy production, a decrease in total energy production and/or substrate availability, and altered phospholipid metabolism.**

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Bipolar disorder remains a prevalent and serious psychiatric disorder.<sup>1</sup> Although a number of pharmacological treatments for bipolar disorder exist, including mood stabilizers such as lithium and valproic acid, none have emerged as singularly effective against all aspects of the illness. Furthermore, the large range of available remedies implies a poor understanding of the underlying pathophysiology of bipolar disorder.

Magnetic resonance spectroscopy (MRS) affords a noninvasive window on *in vivo* brain chemistry and, as such, provides a unique opportunity to gain insight into the biochemical pathology of bipolar disorder. Studies utilizing proton ( $^1\text{H}$ ) MRS have identified changes in cerebral concentrations of *N*-acetyl aspartate (NAA), glutamate/glutamine (Glx), choline (Cho)-containing compounds, myo-inositol (mI), and lactate in bipolar subjects compared to normal controls, while studies using phosphorus ( $^{31}\text{P}$ ) MRS have examined additional alterations in levels of phosphocreatine (PCr), phosphomonoesters (PMEs), and intracellular pH (pHi). However, published reports have generally focused on only one such abnormality at a time, and there remains a lack of coherent models to unify this wide variety of biochemical findings.

It is our hypothesis that the majority of MRS findings in bipolar subjects can be fit into a more cohesive bioenergetic and neurochemical model of bipolar illness that is both novel and yet in concordance with findings from complementary methodological approaches. In this review, we propose a hypothesis of mitochondrial dysfunction in bipolar disorder that involves impaired oxidative phosphorylation, a resultant shift toward glycolytic energy production, a decrease in total energy production and/or substrate availability, and altered phospholipid metabolism (Figure 1). This new understanding of bipolar illness as a matter of possible mitochondrial dysfunction has the potential both to lead to more sophisticated diagnostic techniques and to point toward innovative and hopefully more effective treatments.

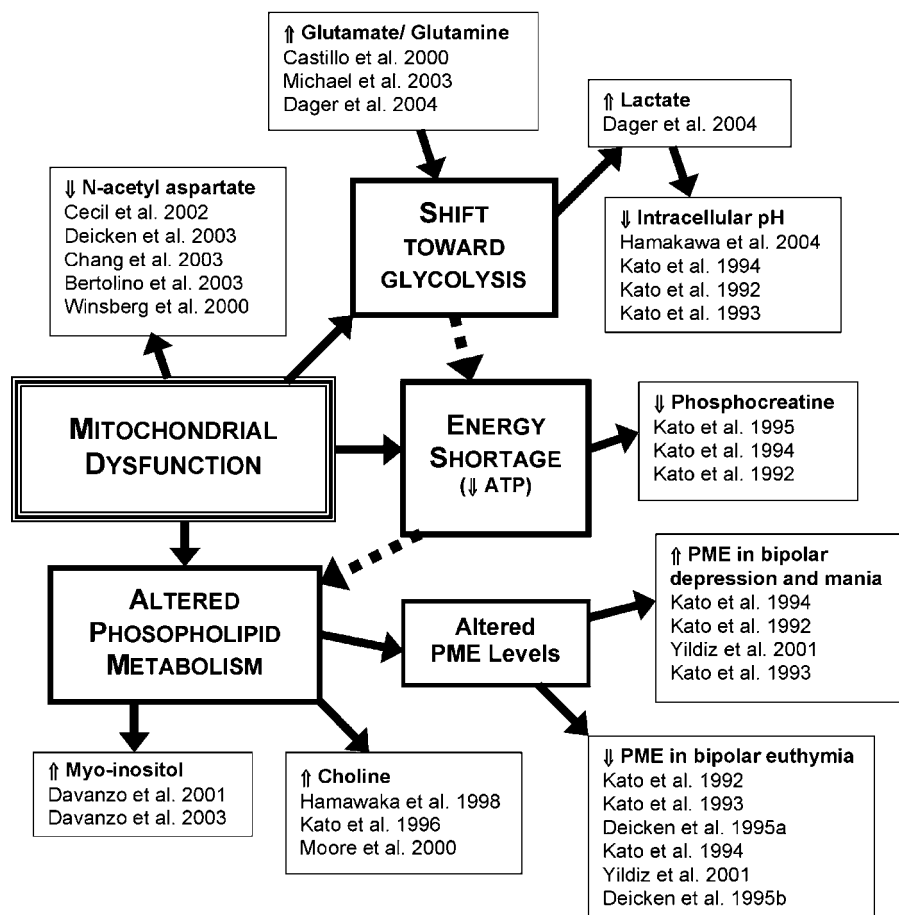
## Reduced *N*-acetyl-aspartate in bipolar disorder: marker of mitochondrial dysfunction

Some of the earliest MRS findings in support of a mitochondrial dysfunction hypothesis of bipolar disorder are the repeated observations of decreased cerebral NAA in bipolar subjects compared to normal controls (Table 1). As the most prominent metabolite peak on the  $^1\text{H}$  spectrum, NAA levels are typically reported as a ratio using the total creatine signal (creatine plus phosphocreatine, Cr + PCr) as an internal reference peak (NAA/Cr + PCr).<sup>2,3</sup> In healthy individuals, NAA is present in the brain at

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**Figure 1** MRS indications of mitochondrial dysfunction in bipolar disorder. Summary of recent MRS research on metabolite alterations in bipolar subjects and how these results may be integrated into a hypothesis of mitochondrial dysfunction.

concentrations of 8–10 mmol/l,<sup>4</sup> and thus follows glutamate as the second most abundant amino acid in the central nervous system.<sup>5</sup> After early research with rat brain mitochondria suggested that NAA is of mitochondrial origin,<sup>6</sup> Truckenmiller *et al*<sup>7</sup> demonstrated that NAA is synthesized in mitochondria by the membrane-bound enzyme *L*-aspartate *N*-acetyltransferase, a catalyst that is found only in the brain (Figure 2). Further studies also indicated that the synthesis of NAA is energy dependent, and stimulated by adenosine diphosphate (ADP).<sup>6</sup>

Despite its prevalence in the human brain, the exact function of NAA remains unclear. Some studies have indicated that NAA may play a role in the provision of carbon for lipid and myelin formation,<sup>8</sup> and other research suggests that NAA may be instrumental in protecting neurons against osmotic stress.<sup>8,9</sup> It has also been suggested that NAA may serve as a readily available precursor of *N*-acetyl aspartyl glutamate (NAAG), a molecule with neurotransmitter-like properties.<sup>10</sup> Most recently, however, Madhavarao *et al*<sup>11</sup> have proposed a model in which the synthesis of NAA is an important component of the ‘mini citric acid cycle.’ In this process, the extra demand for ATP

in neurons is largely met by the oxidation of glutamate via the aspartate aminotransferase pathway. By converting one of the products of this cycle, aspartate, into NAA, NAA biosynthesis is thought to help steer the reaction toward continuing energy production. Additionally, NAA is able to substitute for citrate as an acetate carrier to the cytoplasm, since citrate is not produced during the mini citric acid cycle. In this way, NAA is hypothesized to play an integral role in the energetics of neuronal mitochondria (Figure 2).<sup>11</sup>

Consequently, although reductions in NAA concentration were formerly thought to indicate neuronal death,<sup>12</sup> recent research has suggested that decreased levels of NAA are more accurately consistent with impaired mitochondrial energy production.<sup>8,13</sup> For example, although traumatic brain injury (TBI) is associated with immediate reductions in NAA, several studies have reported that these levels appear to recover significantly with time, which could not occur if NAA levels were decreased by cell death alone.<sup>13,14</sup> In fact, research on cerebral injury by Demougeot *et al*<sup>15</sup> has indicated that cellular dysfunction can actually cause greater measurable reductions

**Table 1** Published MRS research on NAA/Cr + PCr and NAA levels in bipolar subjects

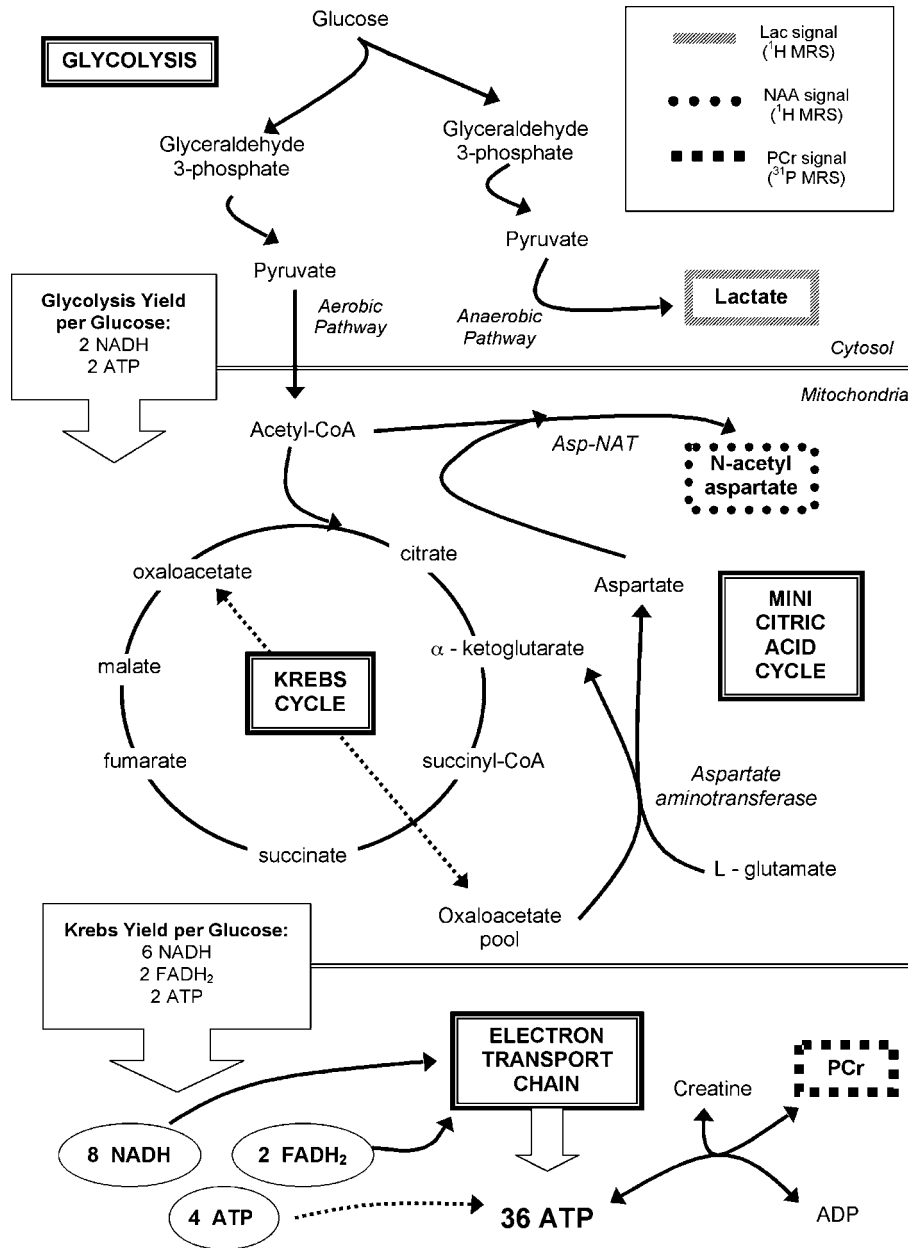
<i>Finding</i>	<i>Populations compared</i>	<i>Author(s)</i>	<i>Subjects' medication status</i>	<i>Reported results</i>
NAA/Cr + PCr; decreased	BP vs NC	Bertolino <i>et al</i> (2003)	6/17 on Li, 2/17 on VPA, 6/17 none, 3/17 on 'other'	BPs with significantly reduced NAA/Cr + PCr bilaterally in hippocampus compared to NCs
NAA/Cr + PCr; decreased	BP children vs NC	Chang <i>et al</i> (2003)	Allowed to continue mood stabilizers and antidepressants; 87% with previous exposure to mood stabilizer	BP children with lower NAA/Cr in right DLPFC
NAA/Cr + PCr; decreased	BP vs NC	Winsberg <i>et al</i> (2000)	N = 20: 10 BP1, 10 BP2; all euthymic and drug free for at least 2 weeks before scan	DLPF NAA/Cr + PCr ratios decreased in both right and left DLPF hemispheres of BPs compared to NCs
NAA; decreased	BPM and Bpmix vs NC	Cecil <i>et al</i> (2002)	17/17 on VPA, 2/17 also on Li	BPs with significantly reduced gray matter (NAA) compared to NCs
NAA; decreased	BPE vs NC	Deicken <i>et al</i> (2003)	7/15 on VPA, 4/15 on Li, 2/15 on 'other,' 3/15 none (one on VPA and Li)	BPs with significantly lower (NAA) bilaterally in hippocampus, even after accounting for tissue type; also significant negative correlation between (NAA) in right hippocampus and illness duration
NAA/Cr + PCr; no change	BPE vs NC	Ohara <i>et al</i> (1998)	7/10 on Li	No significant difference in basal ganglia NAA/Cr between BPs and NCs
NAA; no change	BP vs NC	Hamakawa <i>et al</i> (1999)	13/23 BPEs on Li, 2/8 BPDs on Li	No difference in (NAA) in frontal lobes of BPs vs NCs
NAA; no change	BP children vs NC	Castillo <i>et al</i> (2000)	1 week medication washout period	(NAA) similar in BP children and NCs
NAA; increased	BPE vs NC	Deicken <i>et al</i> (2001)	5/15 on Li, 7/15 on VPA, 1/15 on 'other,' 2/15 none	BPs with significantly higher (NAA) bilaterally in thalamus; no indication of association between (NAA) and illness duration or tissue type

MRS: magnetic resonance spectroscopy; NAA: N-acetyl aspartate; Cr: creatine; PCr: phosphocreatine; BP: bipolar; BPE: bipolar euthymic; BPD: bipolar depressed; BPM: bipolar manic; Bpmix: bipolar mixed; NC: normal control; Li: lithium; VPA: valproic acid or derivative thereof; DLPFC: dorsolateral prefrontal cortex; BP1: bipolar type I; BP2: bipolar type II.

in NAA than neuronal loss. Abnormally low ratios of NAA/Cr + PCr have been observed in patients with a number of mitochondrial encephalomyopathies,<sup>16</sup> and studies using mitochondrial respiratory chain inhibitors have also shown that reductions in NAA are correlated with decreases in O<sub>2</sub> consumption and ATP production.<sup>7,14,17</sup> When taken together, these studies suggest that NAA levels are closely related

to mitochondrial energy metabolism, and thus may serve as a measure of mitochondrial function.<sup>8,17,18</sup>

A number of <sup>1</sup>H MRS studies have demonstrated reduced NAA levels in patients with bipolar disorder compared to normal controls<sup>2,5,19–21</sup> (Table 1). Although the use of Cr as a reference peak has been criticized due to observations of alterations in this signal following treatment with lithium and/or



**Figure 2** Aspects of mitochondrial function visible by MRS. A summary of mitochondrial function in the neuron. Compounds visible by MRS are identified by colored borders.

sodium valproate,<sup>22</sup> findings of decreased NAA/Cr + PCr ratios in bipolar subjects have been corroborated by observations of similarly significant reductions in NAA levels measured relative to voxel H<sub>2</sub>O levels.<sup>5,19</sup> Furthermore, a number of studies have noted negative correlations between NAA/Cr + PCr or NAA levels and illness duration,<sup>2,5,20</sup> which implies that reductions in NAA levels in bipolar subjects may become more pronounced with time. If NAA concentration is interpreted as a measure of mitochondrial function, the collected findings of decreased NAA in bipolar subjects compared to normal controls serve as

support for a theory of mitochondrial dysfunction in bipolar illness.

Researchers have also investigated whether observations of reduced NAA in bipolar subjects may be due to various treatments for the disorder, in particular lithium administration. However, several trials have suggested that lithium administration actually increases NAA levels in the brain<sup>23–25</sup> (Table 2). A number of <sup>1</sup>H MRS studies have reported increased NAA/Cr + PCr and absolute NAA levels associated with lithium administration not only in bipolar subjects,<sup>23–25</sup> but also in healthy controls.<sup>24</sup>

**Table 2** Published MRS research on NAA/Cr + PCr and NAA levels in bipolar subjects: effect of Li administration

<i>Finding</i>	<i>Populations compared</i>	<i>Author(s)</i>	<i>Subjects' medication status</i>	<i>Reported results</i>
NAA; increased; Li	BP and NC after Li vs before Li	Moore <i>et al</i> (2000)	21 subjects: 12 drug-free BP and nine NC	(NAA) increased in all brain regions investigated (frontal, temporal, parietal, and occipital lobes) with Li administration in both BPs and NCs
NAA; increased; Li	BP with Li vs NC	Sharma <i>et al</i> (1992)	All BP on Li	BPs treated with Li had elevated NAA/Cr + PCr in basal ganglia compared to NCs
NAA/Cr + PCr; increased; Li	BPE with Li vs NC	Silverstone <i>et al</i> (2003)	Part I: 14 on Li, 11 on VPA; part II: all nine on VPA	BPs treated with Li had significant increase in (NAA), but not BPs treated with VPA or NCs
NAA; no change; Li	NC with Li vs NC without Li	Brambilla <i>et al</i> (2004)	4 weeks Li administration	Li administration in NCs caused no significant differences in (NAA)

See Table 1 footnote for abbreviations.

Even in contrast with these results, studies led by Brambilla *et al*<sup>26</sup> in healthy individuals and by Friedman *et al*<sup>27</sup> in bipolar subjects have found that lithium administration does not appear to be associated with significant differences in NAA concentration. Thus, there remains a lack of MRS evidence that lithium administration is associated with the reduced NAA levels repeatedly observed in bipolar subjects. Consequently, it is more probable that these reports may reflect an underlying—and possibly mitochondrial—pathophysiology to bipolar illness.

It is important to note that decreased cerebral NAA levels in bipolar patients have not been observed in all MRS investigations. Unaltered NAA levels in bipolar subjects have been observed by Ohara *et al*<sup>28</sup> in the lenticular nuclei, by Hamakawa *et al*<sup>29</sup> in the frontal lobes, and by Castillo *et al*<sup>30</sup> in bipolar children (Table 1). However, seven of the 10 bipolar subjects studied by Ohara *et al*<sup>28</sup> and 13 of the 23 studied by Hamakawa *et al*<sup>29</sup> were undergoing lithium treatment, while the children studied by Castillo *et al*<sup>30</sup> were only given a 1 week medication washout period. Since there is evidence that lithium administration may increase NAA levels in bipolar subjects,<sup>24</sup> it is possible that the results of these three studies were confounded by the history of lithium treatment in their subject populations. Additionally, a further seven of the subjects in the study by Hamakawa *et al*<sup>29</sup> were receiving other pharmacological treatments; only three bipolar subjects were effectively medication free. Consequently, it is also possible that the unaltered NAA levels observed in these studies were due to the normalizing effects of these unidentified treatments.

In contrast with the majority of published MRS research on bipolar disorder, one study by Deicken *et al*<sup>31</sup> reported increased thalamic NAA in male bipolar subjects compared to normal controls. However, all but two of the bipolar subjects in this study were taking maintenance medications such as lithium and divalproex, which may have contributed to the observed increases in NAA.<sup>24,31</sup> Furthermore, since positron emission tomography (PET) studies have reported increases in the concentration of thalamic monoaminergic synaptic terminals in bipolar patients,<sup>32,33</sup> it is possible that Deicken *et al*'s<sup>31</sup> observations of increased thalamic NAA were due to increased synaptic density rather than metabolic abnormality. Similarly, with regard to the findings of Ongur *et al*<sup>34</sup> of regionally reduced glial cell number and density in patients with bipolar disorder, it is also possible that Deicken *et al*'s<sup>31</sup> findings may be due to glial cell hypoplasia in the thalamus. Further studies are required to fully evaluate these possibilities.

As described, findings of decreased NAA in bipolar disorder have been replicated across numerous subject groups by a variety of different research teams (Tables 1 and 2). Together, these studies provide intriguing support for the possibility of mitochondrial involvement in the pathophysiology of bipolar disorder.

### Mitochondrial dysfunction in bipolar disorder: evidence for a glycolytic shift

MRS research has also provided insight into the possible nature of mitochondrial impairment in bipolar disorder. Specifically, findings of both

**Table 3** Published MRS findings of reduced pHi in bipolar subjects

<i>Finding</i>	<i>Populations compared</i>	<i>Author(s)</i>	<i>Subjects' medication status</i>	<i>Reported results</i>
pHi; decreased	BPE vs NC	Hamakawa <i>et al</i> (2004)	8/13 on Li, 1/13 on VPA, remaining on 'other'	BPEs with significantly reduced pHi in basal ganglia by both localized and nonlocalized <sup>31</sup> P-MRS; reflects altered metabolism at cellular rather than regional level
pHi; decreased	BPE vs NC and BPD vs BPE	Kato <i>et al</i> (1992)	(Unknown)	pHi significantly lower in BPEs than in NCs; however, BPs with significantly increased pHi during depressive state compared to euthymic state
pHi; decreased	BPE vs NC	Kato <i>et al</i> (1993)	(Unknown)	BPMs with significantly higher pHi than BPEs; pHi lower for BPEs than for NCs; pHi in BPs not correlated to brain Li concentrations
pHi; decreased	BPI and BPII vs NC	Kato <i>et al</i> (1994b)	All BPs allowed to take any kind of medication	pHi of BP2s similar to NCs, but BP1s with significantly lower pHi than NCs
pHi; decreased	BPE vs NC	Kato <i>et al</i> (1998)	BPs drug free for at least 10 days; 6/7 previously received Li therapy	pHi significantly lower in drug-free bipolar patients compared to NCs

See Table 1 footnote for abbreviations. pHi: intracellular pH.

decreased pHi and increased levels of lactate in bipolar subjects suggest a shift away from oxidative phosphorylation toward glycolysis, thus reducing efficiency and reducing total energy output (Figure 2).

#### *Decreased intracellular pH in bipolar disorder*

As reported by Kato and co-workers, at the present time, reduced pHi has only been observed in pathological states that are known to arise from ischemic insult to the brain, including white matter hyperintensities,<sup>35</sup> acute stroke,<sup>36</sup> and subarachnoid hemorrhage.<sup>37</sup> Additionally, recent research has confirmed that mitochondrial dysfunction plays a central role in ischemic injury, especially in cases of reperfusion following cerebral ischemia.<sup>38–44</sup> Consequently, findings of decreased pHi in bipolar subjects compared to normal controls suggest that impaired mitochondrial function may be an integral component of bipolar illness.

<sup>31</sup>P MRS research has not only consistently reported reduced pHi in bipolar subjects, but has also suggested that this alteration may be state dependent (Table 3). Several studies have found significantly reduced pHi in both the basal ganglia<sup>45</sup> and whole brain<sup>45–49</sup> of bipolar subjects in the euthymic state. However, some of these same studies also indicated that bipolar subjects in the depressed<sup>47</sup> or manic

states<sup>46</sup> had significantly higher pHi than euthymic patients. Furthermore, since pHi has been shown to be significantly correlated with duration of lithium treatment,<sup>50</sup> and lower pHi to be associated with a better response to lithium,<sup>51</sup> it is unlikely that reduced pHi in euthymic bipolar subjects is due to lithium administration. Instead, Kato and Kato<sup>52</sup> have hypothesized that the decreased pHi observed in euthymic bipolar subjects is a trait of the illness itself. Specifically, these researchers have suggested that manic and depressive states may be at least partially caused by the brain's attempts to correct this pH imbalance by causing an overactivation of monoaminergic systems, which has been known to increase pHi at least in hippocampal neurons.<sup>53</sup> Such a theory would also thus explain the more normalized pHi of bipolar patients in the manic and depressive states.<sup>52</sup>

#### *Elevated lactate in bipolar disorder*

Reductions in cerebral pHi such as those observed in bipolar subjects have also frequently been linked to increased levels of lactate, a condition that is known to result from mitochondrial dysfunction. When mitochondrial function is inhibited, rendering the respiratory chain of cellular metabolism unavailable, the only mode of energy production available to the cell is anaerobic glycolysis. During this process,

**Table 4** Published MRS research on glutamate/glutamine (Glx) levels in bipolar subjects

<i>Finding</i>	<i>Populations compared</i>	<i>Author(s)</i>	<i>Subjects' medication status</i>	<i>Reported results</i>
Glx; increased	BP children vs NC	Castillo <i>et al</i> (2000)	1 week medication washout period	Children with BP had elevated levels of Glu/Gln in both frontal lobes and basal ganglia in comparison with NCs
Glx; increased	BP1 and BP2 vs NC	Dager <i>et al</i> (2004)	All patients 'medication free'	BPs with elevated gray matter lactate and Glx compared to NCs
Glx; increased	BPM vs NC	Michael <i>et al</i> (2003)	6/7 drug naïve, 1/7 on Li	BPMs with significantly elevated Glx levels in the left DLPFC compared to NCs

See Table 1 footnote for abbreviations.

pyruvate is used as a hydrogen acceptor to recover NAD from NADH, thus converting pyruvate into lactate (Figure 2). An increase in lactate concentration therefore suggests an increase in the rate of glycolysis, and consequently also implies an inhibition of the mitochondrial oxidative phosphorylation process; lactate is known to accumulate only when oxidative phosphorylation is unable to meet energy requirements and the cell is forced to rely on the glycolytic process.<sup>54</sup> As a result, elevated lactate levels are commonly used to diagnose and confirm mitochondrial disorders, particularly those that affect the central nervous system.<sup>16,55–57</sup> Additionally, animal studies have confirmed that significant increases in brain lactate, as well as decreases in pH<sub>i</sub>, occur as a result of induced, isolated mitochondrial failure.<sup>58</sup> In one study, in which Clausen *et al*<sup>58</sup> used cyanide to induce mitochondrial failure in the feline brain, an analysis of corresponding extracellular lactate concentrations and brain tissue pH suggests a logarithmic relationship between the two measurements ( $pH = 7.949 - 0.138(\ln[\text{Lac}])$ ,  $p(b_0) = 0.0258$ ,  $p(b_1) = 0.1921$ ). Thus, it is possible that seemingly distinct findings of increased lactate and decreased pH<sub>i</sub> are linked by a common cause of mitochondrial dysfunction.

There have been few MRS studies of lactate in bipolar subjects to date, most likely due to the relatively low concentration of lactate in the human brain (<0.7 mM) as well as difficulties in distinguishing the lactate signal from that of overlapping lipids and macromolecules.<sup>59</sup> However, a recent <sup>1</sup>H MRS study by Dager *et al*<sup>60</sup> found elevated lactate levels in the gray matter of medication-free bipolar subjects when compared to normal controls. Furthermore, according to the logarithmic relationship suggested by Clausen *et al*'s<sup>58</sup> data of induced mitochondrial failure in felines, the 0.22 mM difference in lactate concentration reported by Dager between bipolar subjects and controls would correspond to a pH difference of

approximately 0.016, which is the exact pH difference observed between these two populations in the most recent study of cerebral pH<sub>i</sub> in bipolar disorder, published by Hamakawa *et al*.<sup>45</sup> These findings thus suggest that the reduced pH<sub>i</sub> observed in bipolar subjects may be the result of increased levels of lactate, which is in direct concordance with the hypothesis of a glycolytic shift in bipolar disorder.

#### *Alterations in the glutamate/glutamine cycle: possible applications to bipolar disorder*

Further insight into the possibility of a glycolytic shift in bipolar disorder has been provided by <sup>1</sup>H MRS studies of the glutamate/glutamine cycle in bipolar subjects. After glutamate is released from nerve terminals, it is taken up by surrounding glial cells and converted to glutamine. This glutamine is then released by the glia, taken up again by the neurons, and converted back into glutamate for further release into the synaptic cleft. A number of investigations have reported increased levels of Glx, the combined signal arising primarily from glutamate and glutamine, in the frontal lobes, basal ganglia, left dorso-lateral prefrontal cortex (DLPFC), and global gray matter of drug-free bipolar subjects<sup>30,60,61</sup> (Table 4). Although the Glx peak is difficult to separate into glutamate and glutamine at the relatively low magnetic field strengths used in human studies,<sup>62</sup> an increased Glx signal is likely to imply alterations in the overall glutamate/glutamine cycle.

Findings of increased Glx in bipolar subjects suggest that the hypothesized glycolytic shift underlying the pathology of bipolar disorder may be linked to some degree of glutamate-induced neuronal hyperactivation. As suggested by Dager *et al*<sup>60</sup>, increased levels of the excitatory neurotransmitter glutamate in the brain would place abnormally large demands on neuronal energy metabolism, similar to but less severe than the excitotoxic mechanism of cell death that occurs during stroke. If cells are unable to meet such

increased energy requirements through mitochondria-based oxidative phosphorylation, rates of glycolysis would increase, thus causing the increased levels of lactate and decreased pHi observed in studies of bipolar disorder<sup>60</sup> (Table 3). However, further research is needed to confirm this particular aspect of the mitochondrial dysfunction hypothesis of bipolar disorder.

Relatively few MRS studies have examined the effect of treatment on Glx levels in bipolar patients. However, work by Friedman *et al*<sup>27</sup> has indicated that lithium administration may have a strong normalizing effect upon Glx levels in bipolar patients, thus precluding any findings of significant differences between bipolar subjects taking lithium and healthy controls. Since the majority of research subjects with bipolar disorder are required to continue maintenance medications for ethical reasons, it is likely that more universal observations of increased Glx in bipolar patients are confounded by the prevalence of lithium and other pharmacological treatments for the illness.

Interestingly, parallel findings of increased glutamate, glutamine, and lactate have lent support to theories of mitochondrial dysfunction in Huntington's disease (HD).<sup>63</sup> Recent research on HD, an autosomal dominant, progressively neurodegenerative disorder, has led to both excitotoxic and energy metabolism theories of pathophysiology.<sup>64</sup> Preclinical studies have suggested that HD can be mimicked in animals with the intrastriatal injection of certain excitotoxins that activate the *N*-methyl-D-aspartate (NMDA) glutamate receptor, as well as by injection of toxins that block mitochondrial oxidative phosphorylation.<sup>65,66</sup> Similar to studies of bipolar disorder, <sup>1</sup>H MRS investigations of HD have observed both elevated Glx/Cr + PCr and lactate in the basal ganglia of HD patients when compared to normal controls<sup>64,67</sup> In fact, a recent study by Jenkins *et al*<sup>63</sup> observed significantly increased levels of glutamine in a transgenic mouse model of HD, and described these results as 'evidence of a profound metabolic defect.' It is thus possible that similar MRS findings in bipolar

subjects may indicate a similar pathology of excitotoxicity and mitochondrial dysfunction.

### Decreased high-energy compounds in bipolar disorder: evidence for impaired energy production

In continuing support of the hypothesis of a glycolytic shift in bipolar disorder, measurements of PCr, the most prominent peak on the <sup>31</sup>P MRS spectrum,<sup>68</sup> have also been found to be altered in bipolar subjects (Table 5). Acting as a reservoir for the generation of ATP, the high-energy compound PCr is synthesized from Cr and ATP by the catalyzing agent creatine kinase (CPK) (Figure 2).<sup>69</sup> During periods of acute neuronal activity, as molecules of ATP are utilized by Na<sup>+</sup>/K<sup>+</sup> ATPase, PCr is rapidly broken down in order to maintain the overall concentration of ATP.<sup>69,70</sup> Although short-term decreases in PCr concentration thus indicate immediate cell activity, as can be observed in episodes of photic stimulation,<sup>71</sup> long-term abnormalities in PCr concentration generally reflect much larger alterations in cellular metabolism, and in particular an insufficient supply of the ATP needed for normal cellular function.<sup>72</sup> In this way, continually decreased levels of PCr are suggestive of hypometabolism, possibly due to mitochondrial dysfunction, and may directly indicate a shift in energy production toward the less-efficient glycolysis.<sup>72,73</sup> Persistently low brain PCr values have been found in patients with a number of mitochondrial disorders, including mitochondrial encephalomyopathy, lactic acidosis and stroke-like episodes (MELAS), Leigh's disease, progressive external ophthalmoplegia (PEO), and Leber's hereditary optic atrophy.<sup>74,75</sup> Reduced PCr concentrations have also been reported in migraine, which has been postulated to be associated with abnormal mitochondrial function.<sup>76,77</sup>

Several <sup>31</sup>P MRS studies have suggested a connection between decreased levels of PCr and bipolar disorder (Table 5). A study by Kato *et al*<sup>78</sup> of bipolar patients across the full range of mood states (depressed, manic, and euthymic) observed significantly

**Table 5** Published MRS findings of decreased PCr concentration in bipolar subjects

Finding	Populations compared	Author(s)	Subjects' medication status	Reported results
PCr; decreased	BP2 vs NC	Kato <i>et al</i> (1994)	All BPs allowed to take any kind of medication	BP2s with significantly lower (PCr) than NCs regardless of mood state
PCr; decreased	BPD, BPE, and BPM vs NC	Kato <i>et al</i> (1995)	Of BPDs: 4/11 on Li, 5/11 none, 2/11 other; of BPEs: 13/17 on Li, 3/17 none, 1/17 other; of BPMs: 7/12 on Li, 2/12 none, 3/12 other	BPDs with significantly decreased PCr in left frontal lobe compared to NCs; PCr in right frontal lobe of BPMs and BPEs decreased compared to NCs; also, PCr negatively correlated with HDRS

See Table 1 footnote for abbreviations. HDRS: Hamilton Depression Rating Scale.

reduced PCr in the left frontal lobe of depressed patients and in the right frontal lobe of manic and euthymic patients, compared to healthy controls. Additionally, PCr levels were found to be significantly and negatively correlated with Hamilton Depression Rating Scale (HDRS) scores.<sup>78</sup> A similar study by Kato *et al*,<sup>49</sup> only 1 year earlier, also reported significantly decreased whole brain PCr in bipolar II patients, but, interestingly, not bipolar I patients, relative to normal controls.

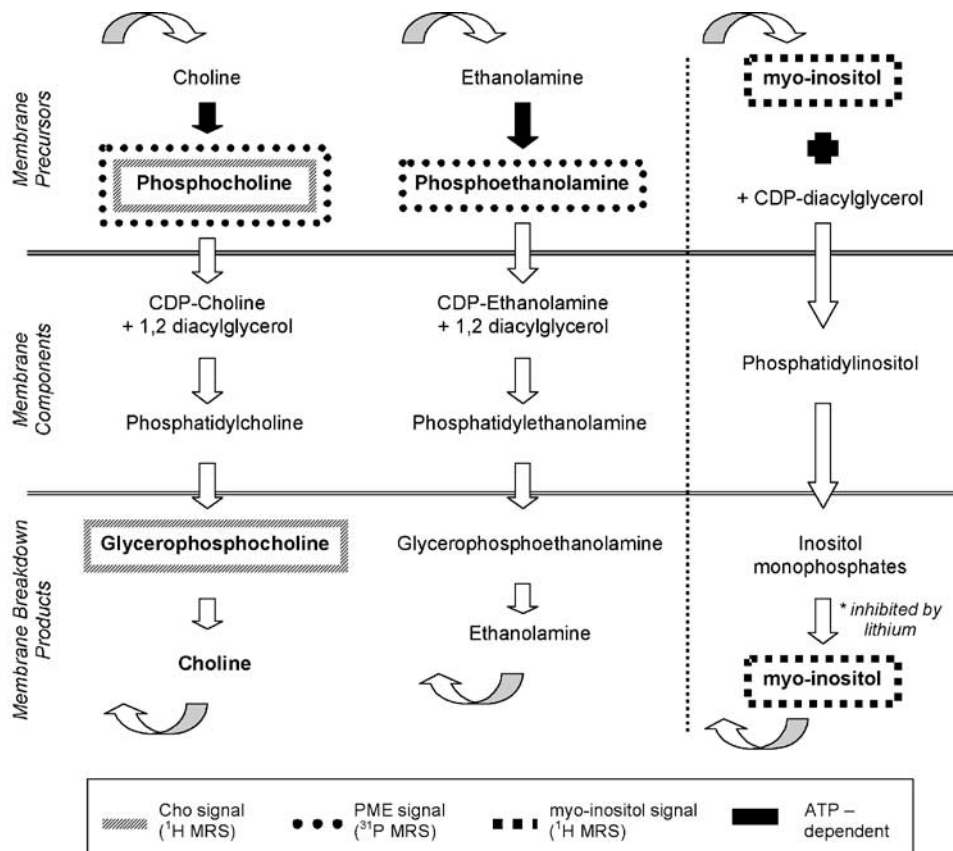
However, MRS research in rat brains has indicated that chronic administration of either lithium or sodium valproate can cause a significant decrease in the <sup>1</sup>H Cr + PCr signal.<sup>22</sup> Consequently, it is possible that the decreased PCr levels observed in these studies were due to the pharmacological effects of these treatments. Of the 40 bipolar patients observed in Kato *et al*'s<sup>78</sup> 1995 study, 24 were reported to be taking lithium, and an additional six to be using other therapies. Similarly, the bipolar II patients in Kato *et al*'s<sup>49</sup> 1994 study were permitted the use of a range of medications. Nonetheless, these alterations of PCr in bipolar disorder are consistent with some degree of mitochondrial involvement in both the illness and its treatment.

### Impaired phospholipid metabolism in bipolar disorder

Further support for a theory of mitochondrial dysfunction in bipolar disorder has arisen from MRS studies of phospholipid metabolism in bipolar subjects. In a normal brain cell, the synthesis and maintenance of the cell membrane require between 10 and 15% of net brain ATP production (Figure 3).<sup>79</sup> If less energy is produced in the cell overall, it is likely that aspects of phospholipid metabolism, including *de novo* phospholipid biosynthesis, would also be impaired. Several MRS studies have indicated that phospholipid metabolism is indeed abnormal in bipolar patients, as evidenced by alterations in levels of Cho, mI, inositol monophosphates, and PME<sub>s</sub> (Tables 6–10). Accordingly, we hypothesize that these alterations are the direct result of energy shortages caused by mitochondrial dysfunction.

#### Elevated total choline in bipolar disorder: evidence for impaired membrane metabolism

One of the most frequently replicated MRS findings in bipolar disorder is that of an increased Cho signal (Table 6). This signal, often referred to as 'total Cho,'



**Figure 3** Aspects of phospholipid metabolism visible by MRS. A partial representation of phospholipid metabolism in the neuron. Compounds visible by MRS are identified by patterned borders. Processes requiring ATP are marked with solid arrows.

**Table 6** Published MRS research on Cho/Cr + PCr and Cho levels in bipolar subjects

<i>Finding</i>	<i>Populations compared</i>	<i>Author(s)</i>	<i>Subjects' medication status</i>	<i>Reported results</i>
Cho/Cr + PCr; increased	BPE and BPD vs NC	Hamawaka <i>et al</i> (1998)	By scan: 12/27 on Li (one also on VPA), 4/27 none, 11/27 on 'other'	Basal ganglia (Cho) significantly higher in BPDs than NCs; BPs in both euthymia and depression had significantly higher Cho/Cr + PCr and Cho/NAA ratios than NCs
Cho/Cr + PCr; increased	BPE vs NC	Kato <i>et al</i> (1996)	10/19 BPs on Li, 9/19 without Li at least 30 days before scan	Cho/Cr + PCr ratio significantly higher in basal ganglia of BPE patients than controls; also, no difference in this ratio between BPE patients treated with Li and those not treated with Li
Cho/Cr + PCr; increased	BP vs NC, and BP with antidepressants vs BP without antidepressants and NC	Moore <i>et al</i> (2000)	5/9 on Li, 4/9 on VPA; 5/9 on antidepressant, 4/9 not on antidepressant	In BPs, depression ratings positively correlated with Cho/Cr + PCr in left cingulate cortex; in right cingulate cortex, Cho/Cr + PCr significantly higher in BPs than NCs; BPs NOT taking antidepressants with significantly higher Cho/Cr + PCr in right cingulate cortex compared with BPs on antidepressants and NCs
Cho/Cr + PCr; increased	BPE vs NC	Lafer <i>et al</i> (1994)	N = 19 BPE: 12/19 on Li and 7/19 not on Li	Higher Cho/Cr in BPEs vs NCs in basal ganglia; no difference in Cho/Cr between patients treated with Li and those without Li
Cho/Cr + PCr; no change	BP vs NC	Bertolino <i>et al</i> (2003)	6/17 on Li, 2/17 on VPA, 6/17 no medication, 3/17 on 'other'	BPs with no significant changes in Cho/Cr + PCr bilaterally in hippocampus
Cho; no change	BPE vs NC	Deicken <i>et al</i> (2003)	7/15 on VPA, 4/15 on Li, 2/15 other, 3/15 none (one on VPA and Li)	No significant differences in (Cho) bilaterally hippocampus between BPEs and NCs
Cho/Cr + PCr; no change	BP vs NC	Winsberg <i>et al</i> (2000)	N = 20: 10 BP1, 10 BP2; all euthymic and drug free for at least 2 weeks at scan	No significant differences in both right and left DLPFC Cho/Cr + PCr in BPs vs NCs
Cho; no change	BP children vs NC	Castillo <i>et al</i> (2000)	1 week medication washout period	Levels of Cho similar in BP children and NCs
Cho; no change	BP children vs NC	Chang <i>et al</i> (2003)	Allowed to continue mood stabilizers and antidepressants; 87% with previous exposure to mood stabilizer	No differences in Cho levels between BP children and NCs

See Table 1 footnote for abbreviations. Cho: choline.

'Cho-containing compounds,' or 'cytosolic Cho,' consists primarily of phosphocholine (PC) and glycerophosphocholine (GPC), with less than 5% of the signal arising from free Cho itself (Figure 3).<sup>59,80</sup> The total observable Cho concentration in the human brain has been measured at 1–2 mM, with approximately 0.6 mM of PC and 1.0 mM of GPC.<sup>81–84</sup>

Cho is required for the synthesis of both the neurotransmitter acetylcholine and the phospholipid phosphatidylcholine (Figure 3). Although acetylcholine is produced only by cholinergic neurons, phosphatidylcholine is produced in all cells as a major membrane constituent.<sup>80,85</sup> Consequently, although small changes in the Cho signal may arise from shuttling between the intracellular and extracellular pools, larger alterations in the Cho signal are generally associated with alterations in membrane synthesis and composition.<sup>59,80</sup> Significant increases in the Cho resonance are commonly observed in neurodegenerative disorders such as Alzheimer's disease and multiple sclerosis (MS), as well as cases of ischemia and head trauma, presumably due to the liberation of Cho-containing compounds during membrane breakdown.<sup>54,59,86</sup> Increased Cho signals can also be associated with cancer, most likely due to the increased cellular density found in tumors.<sup>54,87</sup>

A number of studies have suggested that increased Cho levels may be linked to mitochondrial dysfunction. Perhaps most notably, a 2000 study by Farber *et al*<sup>88</sup> sought to test the hypothesis that the increased Cho signal observed in Alzheimer's disease is due to accelerated phospholipid turnover ultimately caused by mitochondrial dysfunction. Normal brain cells were treated with the mitochondrial uncoupler carbonyl cyanide *m*-chlorophenylhydrazone (CCCP) in the presence of glucose, leaving glycolysis as the only mode of energy production. Enhanced phospholipid turnover was confirmed by the observation of an apparent acceleration of the first two enzymatic steps of phosphatidylcholine synthesis, which resulted in an accumulation of CDP-choline and subsequent lack of diacylglycerol, as well as an increased accumulation of the membrane breakdown product GPC.<sup>88</sup> Although further research is required to elucidate the exact mechanisms that underlie these findings, Farber *et al*<sup>88</sup> did conclude that 'inhibitors of mitochondrial oxidative phosphorylation cause a remarkable acceleration of phosphatidylcholine turnover.'

Several <sup>1</sup>H MRS studies have reported elevated Cho/Cr + PCr ratios and Cho concentrations in bipolar patients compared to healthy controls, particularly in the basal ganglia (Table 6). However, additional MRS investigations of both the hippocampus,<sup>5,21</sup> and DLPFC<sup>2</sup> have reported finding no significant differences in Cho/Cr + PCr or absolute Cho levels between bipolar subjects and controls. Studies of children with bipolar disorder have similarly noted no significant differences in Cho concentrations between bipolar and control subjects.<sup>20,30</sup> Nonetheless, it remains possible that findings of increased Cho

concentration in bipolar subjects may be indicative of impaired phospholipid metabolism and thus also of mitochondrial dysfunction.

Although studies of human erythrocytes have indicated that lithium exerts a strong and specific inhibitory effect on human Cho transport and, thus, substantially elevates erythrocyte Cho concentrations,<sup>89</sup> several MRS studies have reported little to no association between elevated brain Cho concentration and lithium treatment (Table 7). A study by Sharma *et al*<sup>23</sup> reported finding higher Cho/Cr + PCr in the basal ganglia region of bipolar subjects taking lithium compared to normal controls, but a more recent study by Wu *et al*<sup>90</sup> contradicted these results by reporting that lithium-treated bipolar patients had significantly lower Cho/Cr + PCr in the temporal lobe than healthy control subjects. Consequently, if lithium administration does exert a significant effect on brain Cho metabolism, this effect remains to be clarified.

#### *Increased myo-inositol in bipolar disorder: further evidence for impaired membrane metabolism*

<sup>1</sup>H MRS research has also reported alterations of the mI signal in bipolar subjects (Table 8). This signal, one of the three most intense resonances in <sup>1</sup>H MRS, consists primarily of the cyclic sugar alcohol mI, with minor contributions (less than 5%) from various inositol sugar phosphates and glycine (Figure 3).<sup>59</sup> In a healthy adult brain, mI concentrations range from 4 to 8 mmol/kg<sub>ww</sub>,<sup>80</sup> but these levels are known to fluctuate more than any other major compounds in the <sup>1</sup>H spectrum; mI concentrations may reach three times the normal adult values in newborn infants and hypernatremic states and have been observed to drop to almost zero in cases of hepatic encephalopathy.<sup>91</sup>

The function of cerebral mI is not entirely understood. mI has been identified as an essential requirement for cell growth, an osmolyte, a storage form for glucose, and a possible glial marker.<sup>92,93</sup> However, mI is widely recognized for its role as precursor to the phospholipid membrane component phosphatidylinositol and as a substrate for the phosphoinositide second-messenger system.<sup>59,91,94</sup> Consequently, alterations in mI levels have the potential to reflect abnormalities in both membrane metabolism and intracellular signaling mechanisms.<sup>59</sup> Decreased levels of mI have been observed in cases of chronic hepatic encephalopathy, hypoxic encephalopathy, stroke, tumor, and hyponatremia,<sup>91,95</sup> while increased concentrations have been observed in conditions such as Alzheimer's disease, diabetes mellitus, recovered hypoxia, hyperosmolar states, and the neonatal brain.<sup>91</sup> Concerning membrane metabolism in particular, abnormally high levels of mI have also been associated with cases of MS plaque, HIV infection, and metachromatic leukodystrophy, and these findings have led some researchers to label mI as a breakdown product of myelin.<sup>91</sup>

In the study of affective disorders, mI has most often been examined with regard to its apparent

**Table 7** Published MRS research on Cho/Cr + PCr and Cho levels in bipolar subjects: effect of Li administration

<i>Finding</i>	<i>Populations compared</i>	<i>Author(s)</i>	<i>Subjects' medication status</i>	<i>Reported results</i>
Cho/Cr + PCr; no change; Li	BPE vs NC	Ohara <i>et al</i> (1998)	7/10 BPEs on Li	No significant difference in basal ganglia Cho/Cr between BPs (most on Li) and NCs
Cho; no change; Li	BP with Li vs NC (without Li)	Stoll <i>et al</i> (1992)	Seven BP on Li, six NC without Li	No significant difference in (Cho) between BP patients on Li and NCs
Cho/Cr + PCr; no change; Li	BP with Li vs BP without Li	Kato <i>et al</i> (1996)	10/19 BPs with Li, 9/19 without Li at least 30 days	No difference in Cho/Cr + PCr between BPEs treated with Li and those not treated with Li
Cho; no change; Li	NC before Li vs NC after Li	Brambilla <i>et al</i> (2004)	4 weeks Li administration in NCs	Li administration in NCs caused no significant differences in the levels of Cho-containing compounds
Cho/Cr + PCr; no change; Li	NC with Li vs NC with placebo	Silverstone <i>et al</i> (1999)	7 days blinded administration of Li or placebo	No significant differences in Cho/Cr + PCr in temporal lobe between NCs on Li and NCs on placebo
Cho/Cr + PCr; no change; Li	BP with Li vs BP without Li	Lafer <i>et al</i> (1994)	<i>N</i> = 19 BPE: 12/19 on Li and 7/19 not on Li	No difference in Cho/Cr between BPs treated with Li and those without Li
Cho/Cr + PCr; increased; Li	BP with Li vs NC	Sharma <i>et al</i> (1992)	All BP on Li	BP patients on Li demonstrated elevated Cho/Cr + PCr in basal ganglia region vs NCs
Cho/Cr + PCr; decreased; Li and VPA	BPEs with Li vs NC	Wu <i>et al</i> (2004)	Part I: 14 BPE on Li, 11 BPE on VPA	Part I: BPEs treated with either Li or VPA had significantly reduced Cho/Cr in temporal lobe compared to NCs

See Table 1 footnote for abbreviations. Cho: choline.

involvement in the mechanism of action of lithium. A number of studies have demonstrated that at therapeutically relevant concentrations, lithium is a potent, noncompetitive inhibitor of inositol-1-phosphatase, an enzyme that normally serves to recycle inositol sugar phosphates back into the free inositol pool. This inhibition leads to an accumulation of inositol-1-phosphate (a component of the PME signal in <sup>31</sup>P MRS) and a simultaneous decrease in levels of mI.<sup>96–98</sup> Since sufficient supplies of mI are necessary both for the re-synthesis of certain membrane components and to maintain the phosphoinositide intracellular signaling system, it has been suggested that lithium achieves its effects through this depletion of the free inositol pool.<sup>99,100</sup>

Several MRS studies have supported this theory with findings of decreased mI levels in subjects undergoing acute lithium administration (Table 8).

As lithium continues to be one of the most common and effective pharmacological treatment for bipolar disorder, MRS measurements of mI levels in bipolar subjects must be considered with lithium administration as a confounding factor; observations of both increased or decreased mI concentrations (as well as those of altered PMEs, due to the possible accumulation of inositol-1-phosphate; see the following section) in bipolar individuals may be due to the effects of lithium treatment rather than the disease itself.

Importantly, however, it is also possible that the effect of lithium on inositol and PME levels may not remain constant with continuous administration. Although several studies have demonstrated that acute lithium administration does initially cause a large increase in PME levels (attributed to increased inositol-1-phosphate) and a decrease in mI,<sup>101</sup> a <sup>31</sup>P study by Renshaw *et al*<sup>102</sup> in cats observed that such

**Table 8** Published MRS research on mI levels in bipolar subjects

<i>Finding</i>	<i>Populations compared</i>	<i>Author(s)</i>	<i>Subjects' medication status</i>	<i>Reported results</i>
mI/Cr + PCr; decreased; Li	BP children vs NC	Davanzo <i>et al</i> (2001)	1 week acute Li administration	Significant reduction in mI/Cr ratio in ACC voxel with acute Li treatment; also significant in Li responders analyzed separately from nonresponders; BPMs with higher mI/Cr than NCs
mI; decreased; Li	BP with Li vs BP without Li	Moore <i>et al</i> (1999)	Medication washout (minimum 2 weeks) and Li administration conducted in a blinded manner	BPD patients had significant decreases in mI levels in right frontal lobe with Li administration; HDRS scores also decreased over the study
mI; no change; Li and VPA	BP with Li or VPA vs NC	Silverstone <i>et al</i> (2002)	Part I: 14 on Li, 11 on VPA; part II: all nine on VPA	No differences in either mI or PME concentrations between NCs and BPs taking either Li or VPA
mI; no change; Li	NC with Li vs NC without Li	Brambilla <i>et al</i> 2004	4 weeks Li administration	Li administration in NCs caused no significant differences in mI levels
mI; increased	BP vs NC and 'intermittent explosive disorder'	Davanzo <i>et al</i> 2003	2/10 BP on stimulants, 2/10 BP on VPA, and 3/10 on other	BPs with significantly higher anterior cingulate mI/Cr + PCr and (mI) than patients with intermittent explosive disorder and NCs
mI; increased; Li	BP with Li vs NC	Sharma <i>et al</i> (1992)	BP on Li	BP patients on Li demonstrated elevated inositol in basal ganglia region vs NCs
mI/Cr + PCr; trend toward increased	BP vs NC	Winsberg <i>et al</i> (2000)	20 BPs: 10 BP1, 10 BP2; all euthymic and drug free for at least 2 weeks at scan	Trend toward higher mI/Cr + PCr in right DLPFC ( $P=0.06$ ) of BPs vs NCs

See Table 1 footnote for abbreviations. PME: phosphomonoester.

increased PME levels subsequently decline after 3 weeks of lithium administration. Consequently, since lithium is frequently used as a long-term treatment regimen for bipolar disorder, it is possible that PME levels in individuals with a history of lithium treatment may have normalized after an initial increase. For example, a 2002 study by Silverstone *et al*<sup>103</sup> found no differences in mI or PME concentrations between bipolar patients under chronic lithium treatment and normal controls, and thus suggested that long-term lithium treatment may normalize the phosphoinositide cycle of individuals with bipolar disorder. Similarly, a study by Brambilla *et al*<sup>26</sup> of healthy controls before and after lithium administration found no significant differences in mI concentration after 4 weeks of lithium treatment.

Because of the widespread use of lithium, there are few MRS data from bipolar subjects that can be analyzed without regard to its effects (Table 8). However, one study by Davanzo *et al*<sup>104</sup> did report increased mI/Cr + PCr levels in the anterior cingulate of children and adolescents with bipolar disorder who had never been treated with lithium, when compared to normal controls. Sharma *et al*<sup>23</sup> also reported findings of increased inositol in the basal ganglia of bipolar patients vs comparison subjects, although patients in this study were not lithium free. Additionally, a study by Winsberg *et al*<sup>2</sup>, in which bipolar patients were reportedly drug free for at least 2 weeks before examination, found a nearly significant trend ( $P=0.06$ ) toward higher levels of mI/Cr + PCr in the right DLPFC

**Table 9** Published MRS findings on PME levels in bipolar subjects: bipolar euthymic vs normal controls

<i>Finding</i>	<i>Populations compared</i>	<i>Author(s)</i>	<i>Subjects' medication status</i>	<i>Reported results</i>
PME; decreased	BPE vs NC	Deicken <i>et al</i> (1995a)	All BP with 1 week washout period	BPs with significantly lower (PME) and significantly higher (PDE) in both left and right frontal lobes in comparison to NCs
PME; decreased	BPE vs NC	Deicken <i>et al</i> (1995b)	All BP with 1 week washout period	BPs with significantly lower (PME) than NCs in both left and right temporal lobes
PME; decreased	BPE vs NC	Kato <i>et al</i> (1992)	(Unknown)	PME levels significantly lower in BPEs than in NCs
PME; decreased	BPE vs NC	Kato <i>et al</i> (1993)	(Unknown)	PMEs significantly lower in BPEs compared to NCs; PME levels not correlated to brain Li concentrations
PME; decreased; Li	BP1(E) vs NC	Kato <i>et al</i> (1994a)	All BPs with at least 2 weeks Li treatment	BP1(E)s with decreased (PME) compared to NCs
PME; decreased	BPE vs NC	Yildiz <i>et al</i> (2001)	Meta-analysis; medication differences not included	PME values of BPEs significantly lower than PME values of NCs
PME; no change	BPE vs NC	Kato <i>et al</i> (1998)	BPs drug free for at least 10 days; 6/7 had previously received Li therapy	No significant difference in (PME) between drug-free BPs and NCs

See Table 1 footnote for abbreviations. PME: phosphomonoester; PDE: phosphodiester.

**Table 10** Published MRS findings on PME levels in bipolar subjects: bipolar depressed/manic vs bipolar euthymic

<i>Finding</i>	<i>Populations compared</i>	<i>Author(s)</i>	<i>Subjects' medication status</i>	<i>Reported results</i>
PME; increased	BPD vs BPE	Kato <i>et al</i> (1992)	(Unknown)	BPs in depressive state with significantly increased PME compared to euthymic state
PME; increased	BPM vs BPE	Kato <i>et al</i> (1993)	(Unknown)	BPMs had significantly higher PME than BPEs; PME levels not correlated to brain Li concentrations
PME; increased	BPD vs BPE	Yildiz <i>et al</i> (2001)	Meta-analysis; medication differences not included	BPDs with significantly higher PME than BPEs
PME; increased; Li	BPM vs BPE and NC	Kato <i>et al</i> (1991)	All BPs treated with Li	Elevated PME in BPM on Li compared to BPE on Li and drug-free NCs

See Table 1 footnote for abbreviations. PME: phosphomonoester.

of bipolar subjects vs controls. Although the confounding effects of lithium cause difficulty in the interpretation of mI levels in bipolar patients, it may yet be argued that elevated mI

levels suggest an increase in membrane breakdown and inhibition of membrane metabolism in bipolar disorder that may be caused by mitochondrial dysfunction.

### *Altered phosphomonoesters: evidence for state-dependent abnormalities of membrane metabolism in bipolar disorder*

Interestingly,  $^{31}\text{P}$  MRS studies of PME levels in bipolar subjects have indicated that some abnormalities in bipolar membrane metabolism may be state dependent, specifically differing during periods of euthymia, mania, and depression (Tables 9 and 10). Similar to the multicomponent Cho signal, the PME signal observed by  $^{31}\text{P}$  MRS is the combined product of multiple compounds. *In vitro* and *in vivo* assays have determined that the most abundant components of the PME signal are the membrane precursors phosphoethanolamine (PE) and PC, but the signal also includes various sugar and inositol phosphates (Figure 3).<sup>105,106</sup>

Because of the multiple membrane metabolites represented in this signal, alterations in PME levels are thought to reflect concurrent alterations in phospholipid membrane metabolism. Specifically, an increase of the PME peak has been suggested to indicate an increased rate of membrane phospholipid turnover.<sup>94</sup> Increased PME levels have been observed in the neonate brain, tumors,<sup>36,107</sup> and the regenerating liver.<sup>108</sup> In contrast, decreased PME levels, possibly reflecting a stagnation or halt of membrane synthesis, have been associated with chronic cerebral infarction,<sup>35</sup> severe demyelinating disorders,<sup>12</sup> anorexia nervosa,<sup>109</sup> and schizophrenia (particularly the negative symptoms thereof).<sup>110,111</sup>

In consideration of the previously noted  $^1\text{H}$  MRS findings of increased Cho and mI in bipolar subjects, it is important to recognize the partial inclusion of these metabolites within the PME peak. Specifically, alterations in the Cho or inositol pathways may also be reflected in alterations of the PME signal, since this peak includes both PC (30%) and inositol monophosphates (less than 5%).<sup>112</sup> However, since these various metabolites are only components of the larger PME resonance, PME measurements may provide independently significant insights into phospholipid metabolism.

Unlike many MRS findings in bipolar patients, research on PME levels in bipolar disorder has indicated the possibility of state-dependent changes in concentration. Specifically, while several studies have observed increased PME signals in bipolar patients in the manic or depressed states compared to those in euthymia, a number of investigations have reported decreased PME levels in euthymic bipolar subjects compared to normal controls (Table 9). Many of these findings were also evaluated in a meta-analysis of  $^{31}\text{P}$  MRS research on bipolar disorder by Yildiz *et al*<sup>113</sup>, which confirmed reports of significantly lower PME levels in euthymic bipolar subjects *vs* normal controls. One 1998 study by Kato *et al*<sup>48</sup> did not find a difference in PME levels between euthymic bipolar subjects and controls, but had an extremely small sample group of only seven subjects. It thus remains possible that observations of decreased PME levels in euthymic bipolar patients reflect a general

inhibition of membrane synthesis that may be related to mitochondrial impairment.

However, when euthymic bipolar subjects are compared to others in the depressed or manic states, MRS research has illustrated a considerably different picture (Table 10). Individual studies by Kato *et al* have reported significantly elevated PME levels in both depressed<sup>47</sup> and manic<sup>46</sup> bipolar subjects compared to those in euthymia, and the later meta-analysis by Yildiz *et al*<sup>113</sup> also found significantly increased PME levels in depressed *vs* euthymic bipolar subjects. Like measurements of altered pHi in bipolar subjects (Table 3), these state-dependent abnormalities in PME concentration may be the result of the brain's attempts to normalize membrane metabolic processes that are impaired due to mitochondrial dysfunction.

As discussed in the previous section, numerous studies have indicated that acute lithium administration is associated with a large increase in PME levels,<sup>101</sup> although it has also been reported that these levels later normalize with continued lithium treatment.<sup>102</sup> Importantly, however, a dual lithium and  $^{31}\text{P}$  MRS study by Kato *et al*<sup>46</sup> found that PME levels in bipolar patients in both the euthymic and manic states did not correlate with brain lithium concentrations. Additionally, in 1991, Kato *et al*<sup>114</sup> found PME levels in manic bipolar patients on lithium to be significantly greater than those of euthymic bipolar patients who were also on lithium. Consequently, Kato's team was led to conclude that 'PME levels in the manic state cannot be attributed solely to elevation of brain lithium concentrations.'<sup>46</sup> Instead, observations of both increased and decreased PME levels in bipolar subjects may yet signify abnormal membrane metabolism and thus further support the hypothesis of mitochondrial dysfunction as an underlying pathology of bipolar illness.

### **Connection to other findings in bipolar research**

Although this review has focused on MRS findings in particular, the hypothesis of mitochondrial dysfunction in bipolar disorder is also supported by research using other methodologies. For example, the possibility of mitochondrial dysfunction can be easily linked to the previously developed and more traditional hypotheses of intracellular signaling alterations as key aspects of bipolar pathophysiology.<sup>1</sup> A number of studies of bipolar subjects have reported various abnormalities of second-messenger systems, including alterations in cyclic AMP (cAMP) signaling,<sup>115</sup> protein kinase C (PKC) signaling,<sup>116</sup> and the overall phosphatidylinositol pathway (reviewed by Soares and Mallinger<sup>117</sup>). In addition, studies have consistently reported that agonist-stimulated calcium response, an important factor in intracellular second-messenger systems, is enhanced in platelets in patients with bipolar disorder (reviewed by Yamawaki *et al*<sup>118</sup>). Given that recent research has demonstrated the important role of mitochondria in

sequestering increased intracellular calcium caused by agonist stimulation (reviewed by Simpson and Russell<sup>119</sup>), it is possible that the mitochondrial dysfunction suggested by MRS research in bipolar disorder is directly related to these previously observed alterations of calcium response and intracellular signaling systems in bipolar patients.

The mitochondrial dysfunction hypothesis of bipolar disorder may also account for the repeated indications of altered cerebral metabolism observed in functional studies of bipolar patients. A number of PET studies have demonstrated that cerebral glucose metabolic rates are significantly decreased in bipolar subjects in depressed or mixed mood states.<sup>120–125</sup> Additional studies have reported that these low metabolic rates also tend to increase with symptom remission<sup>126</sup> or a shift to a more euthymic or hypomanic state.<sup>125</sup> PET studies of bipolar individuals in the manic state have reported decreased regional cerebral blood flow (rCBF) in the right ventral temporal lobe,<sup>127</sup> orbitofrontal cortex,<sup>128</sup> and frontal regions compared to normal controls,<sup>129</sup> although a 2000 study by Blumberg *et al*<sup>130</sup> found manic patients to have significant higher rCBF in the left dorsal anterior cingulate cortex (ACC) and left head of the caudate when compared to a separate sample of euthymic bipolar patients. Although PET research cannot identify the etiology of these metabolic differences in bipolar patients, it is possible that they are related to the mitochondrial dysfunction suggested by MRS studies.

Furthermore, investigations of mitochondrial genetics have also found significant evidence of abnormal mitochondrial function in bipolar disorder. Using a quantitative polymerase chain reaction (PCR) method, a 1997 study by Kato *et al*<sup>131</sup> found a significantly higher ratio of deleted to wild-type mitochondrial DNA (mtDNA) in the cerebral cortex of patients with bipolar disorder compared to age-matched controls. After 3 years, Kato *et al* also reported a significantly higher rate of the 5178C mtDNA genotype, known to be associated with an increased risk and earlier onset of both Alzheimer's and Parkinson's disease,<sup>132</sup> in bipolar patients compared to controls.<sup>133</sup> In addition, bipolar patients with the 5178C genotype exhibited significantly lower brain pH than patients with the 5178A genotype,<sup>133</sup> which suggests a possible connection between abnormal mtDNA and the reduced pH levels consistently reported in bipolar patients (Table 3). Finally, in a 2004 study of mRNA expression in the hippocampus of patients with bipolar disorder and schizophrenia compared to healthy controls, Konradi *et al*<sup>134</sup> found bipolar subjects to have significantly decreased expression of nuclear messenger RNA coding for mitochondrial proteins. Specifically, patients with bipolar disorder were characterized by 'a pronounced and extensive decrease in the expression of genes regulating oxidative phosphorylation and the ATP-dependent process of proteasome degradation.'<sup>134</sup> Especially when viewed in light of the MRS evidence

presented in this review, these findings considerably strengthen the hypothesis of mitochondrial dysfunction in bipolar disorder.

## Conclusion

Although MRS research on bipolar disorder, to date, has largely focused on individual brain metabolite abnormalities, it is our hypothesis that the compilation of these single-compound findings illustrates an intriguing pattern of mitochondrial dysfunction in bipolar illness (Figure 1). The integration of such a large and seemingly disparate collection of MRS findings in bipolar subjects into a single theory of mitochondrial dysfunction has enormous potential for advancing the study of bipolar illness, particularly in terms of treatment. Investigations of mitochondria-based neurological disorders, including HD, Parkinson's disease, amyotrophic later sclerosis (ALS), and mitochondrial cytopathies, have led to the development of a number of therapies that target cellular energy dysfunction.<sup>135</sup> If mitochondrial dysfunction is involved in its underlying pathology, such therapies may also prove beneficial in the treatment of bipolar disorder. Studies of HD, an illness with an MRS profile similar to that of bipolar disorder (increased concentrations of lactate, glutamate, and Cho, as well as decreased NAA levels<sup>136</sup>), have found that Cr supplementation in particular can significantly increase brain concentrations of Cr and ATP to normal levels, thus exerting a neuroprotective effect and slowing the process of brain atrophy.<sup>137,138</sup> Additional studies of Parkinson's disease and TBI have also investigated the potential use of compounds that may improve cellular energy function, such as coenzyme Q<sub>10</sub>, *Ginkgo biloba*, nicotinamide, and lipoic acid, but evidence as to the therapeutic efficacy of these treatments remains extremely limited.<sup>135,139,140</sup> Yet if bipolar disorder can be viewed in terms of its possible basis in mitochondrial dysfunction, such therapies have the potential to ameliorate aspects of the disease that are only minimally addressed by current treatment methods.

Furthermore, an understanding of bipolar illness in terms of mitochondrial dysfunction may greatly affect the usage of those treatments currently available. A number of MRS studies have indicated that lithium, the most commonly used pharmacotherapy for bipolar disorder,<sup>1</sup> may increase NAA levels in bipolar subjects (Table 1b) and thus ostensibly improve mitochondrial function, but a study by Silverstone *et al*<sup>25</sup> reported that this effect is not associated with the administration of sodium valproate. In fact, research on the mechanism of action of valproate has suggested that the drug has a high degree of interference with cellular energy metabolism, and may impair brain fuel utilization (reviewed by Bolaños and Medina<sup>141</sup>). Consequently, if bipolar disorder does stem from some degree of mitochondrial dysfunction, it may be important to investigate whether the augmentation of valproate administration

with agents that counteract the drug's effects on cellular energetics would improve the clinical efficacy of the treatment regimen as a whole. In addition, research on neuroleptics has indicated that typical and atypical antipsychotics have substantially different effects on respiratory enzyme activity levels.<sup>142</sup> Although typical agents inhibit Complex I of the electron transport chain<sup>143,144</sup> and thus may cause significant reductions in synaptic ATP synthesis and mitochondrial respiration,<sup>145</sup> atypical agents have comparatively no effect on electron transfer activities.<sup>142</sup> Consideration of the possible mitochondrial factors underlying bipolar disorder may thus prove valuable in the choice and prescription of such antipsychotic treatments.

A recent feature review published in *Molecular Psychiatry* proposes that novel treatments developed specifically for bipolar disorder will arise from either '(1) understanding the mechanism of action of current medications and thereafter designing novel drugs that mimic these mechanism(s)' or '(2) basing medication development upon the hypothetical or proven underlying pathophysiology of bipolar disorder.'<sup>146</sup> We feel that the hypothesis of mitochondrial dysfunction in bipolar disorder, as supported by evidence from MRS, has the potential to fulfill both these objectives. It remains our hope that, in the future, a better understanding of both current therapies and the underlying pathophysiology of bipolar illness may lead to the development of more sophisticated treatments, and consequently improve the quality of life of thousands of affected individuals.

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