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Mitochondrial Dysfunction in Bipolar Disorder: Lessons from Brain Imaging and Molecular Markers

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Abstract

Bipolar disorder (BD) is a chronic major mental illness characterized by extreme mood episodes, cognitive impairment, and high rates of disability. Several lines of evidence suggest that BD may be associated with abnormalities in mitochondrial function. Here we critically review findings from brain imaging and from preclinical studies that investigated markers of energy metabolism in BD. Research with postmortem brain and peripheral tissue revealed changes in size and distribution of mitochondria, as well as decreased mitochondrial electron transport chain function, increased oxidative stress, and increased lipid and protein damage. PET imaging studies revealed decreased glucose metabolism in sub-areas of the prefrontal cortex, amygdala, and hippocampus structures in BD. On the other hand, increased lactate levels in BD have been found in cerebrospinal fluid and in gray matter by magnetic resonance spectroscopy, which suggest that distinct pathophysiological processes may be region-specific. Resting state fMRI studies have demonstrated decreased functional connectivity between fronto-limbic circuits. In conclusion, these results support the hypothesis of mitochondrial dysfunction in BD and suggest that BD is associated with decreased energy production and a shift towards anaerobic glycolysis. Such changes in energy metabolism can potentially decrease cell plasticity and ultimately disrupt brain circuits associated with mood and cognitive control.

Key words: Mitochondrial dysfunction, bipolar disorder, PET, biomarkers.

Título: Disfunción mitocondrial en el trastorno bipolar: lecciones de las imágenes cerebrales y los marcadores moleculares

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Resumen

El trastorno bipolar (TB) es una enfermedad mental crónica grave caracterizada por episodios de ánimo extremo, trastornos cognitivos y altas tasas de discapacidad. Varias líneas de evidencia sugieren que el TB puede estar asociado con anormalidades en la función mitocondrial. Aquí analizamos críticamente los hallazgos de las imágenes cerebrales y de los estudios preclínicos que han investigado los marcadores del metabolismo de energía en TB. Las investigaciones post mórtem basadas en tejidos cerebrales y tejidos periféricos revelaron cambios en el tamaño y en la distribución de las mitocondrias, además de una disminución en la funcionalidad de la cadena de transporte de electrones de las mitocondrias, un mayor estrés oxidativo y mayores daños lipídicos y proteínicos. Estudios con imágenes TEP revelan un metabolismo de glucosa disminuido en las subáreas de la corteza prefrontal, la amígdala y el hipocampo en TB. Por otro lado, se han hallado concentraciones mayores de lactato en TB en el líquido cefalorraquídeo cerebral y en la materia gris utilizando la espectroscopia con resonancia magnética, lo cual sugiere que los procesos fisiopatológicos individuales pueden ser específicos de las distintas regiones. Los estudios con resonancias magnéticas funcionales han demostrado una menor conectividad funcional entre los circuitos frontolímbicos. En conclusión, estos resultados apoyan la hipótesis de una disfunción mitocondrial en el TB y sugieren que el TB está asociado con una menor producción de energía y un cambio hacia la glicólisis anaeróbica. Estos cambios en el metabolismo energético pueden disminuir potencialmente la plasticidad celular y, en últimas, perturbar los circuitos cerebrales asociados con el estado de ánimo y el control cognitivo.

Palabras clave: Disfunción mitocondrial, trastorno bipolar, tomografía por emisión de positrones, biomarcadores.

Introduction

Bipolar disorder (BD) is a chronic and disabling major mental illness characterized by episodic disturbances in mood reactivity (e.g. mania, depression) and cognitive impairment. Even though BD is associated with structural and functional brain changes, the fact that BD is also associated with increased mortality due to general medical conditions such as heart disease and cancer (1), as well as higher risk for metabolic syndrome, suggests that BD is a systemic rather than a brain-limited disorder (2). Over the past decade, there has been converging evidence supporting the hypothesis of mitochondrial dysfunction as a key element in the pathophysiology of BD (3-5). The brain metabolizes approximately 20% of all of the body's oxygen because it works under a constant high energy demand. Considering that most of the energy produced in the brain is used to replenish the ATP consumed by the Na-K-ATPase pump and in the production/metabolism of neurotransmitters, mitochondrial dysfunction may disrupt the membrane's ionic gradient and the glutamatergic clearance, thereby leading to abnormal neuronal firing. Furthermore, persistent glutamatergic activity may lead to a state of excitotoxicity and potentially cell death.

Here we critically review studies conducted with postmortem brain tissue, peripheral blood and studies in vitro that provide evidence of mi-

tochondrial dysfunction in BD, as well as data from positron emission tomography, resting state functional magnetic resonance imaging and magnetic resonance spectroscopy that assessed energy metabolism in individuals with BD in vivo.

Molecular Markers of Mitochondrial Dysfunction

Evidence of mitochondrial dysfunction in BD is suggested by altered mitochondrial morphology, impaired brain energy metabolism, altered mitochondria-dependent Ca2+ signaling, the effects of mood stabilizers on mitochondria, increased mitochondrial DNA deletion in the neural tissue of BD patients, and the association of mitochondrial DNA mutations and/or polymorphisms with BD (4-6).

Morphological changes of mitochondria have been observed in the central nervous system (CNS) as well as in peripheral tissue in individuals with BD. Mitochondria from patients with BD exhibited size and distributional abnormalities compared with psychiatrically- healthy age-matched controls. Specifically, in the prefrontal cortex, individual mitochondria profiles had significantly smaller areas in individuals with BD (7). In two peripheral cell types (fibroblasts and lymphocytes) the mitochondria from BD individuals exhibited alterations in distribution and morphology, showed by dense or bulky networks with perinuclear clustering profile. This study was the first to demonstrate that brain cortical and also peripheral cells from BD patients display abnormalities in the morphology (size and shape) and in the intracellular distribution of mitochondria. Given that abnormal mitochondrial morphology is linked to altered energy metabolism, changes in mitochondrial size and distribution may lead to energy deficits and, therefore, may have consequences for cell plasticity, resilience, and survival in patients with BD.

Abnormalities in mitochondrial function have also been observed in BD. A recent postmortem study reported a reduction in the activity of the complex I of the mitochondrial electric transport chain (ETC) in the prefrontal cortex in BD, but not in schizophrenia (SZ) or major depressive disorder (MDD), and this reduction was associated with increased protein oxidative damage (8). Prolonged impairment in oxidative phosphorylation activity causes accumulation of lactate and unprocessed glucose. Consistent with this, a study found increased lactate in the cerebrospinal fluid of BD patients, which indicates a shift from mitochondrial respiration to anaerobic glycolysis (9). Some data suggest that cellular response to metabolic stress may be impaired in BD, and such impairment may be closely linked to mitochondrial function. For instance, the molecular response to glucose deprivation in lymphocytes of BD patients was significantly re-

duced as compared to cells of normal controls (10). Microarray analysis of the peripheral blood mononuclear cells revealed that control subjects upregulated expression of ETC genes in response to glucose deprivation, while cells from BD patients failed to show any changes in mitochondrial gene expression (10). Together, these studies suggest that alterations in the ETC within the mitochondria may be associated with a shift from oxidative phosphorilation to anaerobic glycolysis in BD. In this context, a preliminary study revealed that the mood stabilizer lithium can increase the activity of mitochondrial ETC complexes I/II and II/III in human brain tissue (11), which suggests that lithium may stabilize mitochondrial function.

Creatine kinase (CK) is a key enzyme involved in the transport of ATP from mitochondria to cytosol. An elegant study conducted by MacDonald et al (2006) found that CK mRNA were downregulated in the hippocampus and dorsolateral prefrontal cortex in BD (12). Considering the central role of CK in the transport of intracellular high-energy phosphates, this study revealed another indicative of altered energy metabolism in specific brain areas in BD. However, one study conducted in rats reported that neither lithium nor valproate were able to reverse or prevent the inhibition of CK activity induced by amphetamine (13).

Several lines of evidence suggest that BD may be associated with altered Ca2+ signaling. Since mitochondria buffer cytosolic Ca2+, abnormal Ca2+ homeostasis may induce detrimental effects on mitochondrial and cell function and viability (14,15). In addition, the interplay between elevated intracellular Ca2+ concentration, reactive oxygen species (ROS) formation, and mitochondrial dysfunction has been long associated with brain pathology (3, 14). Studies with peripheral blood found elevated Ca2+ levels in platelets and lymphocytes of BD patients (16-18). Also, lymphoblastoid cell lines derived from BD patients showed higher Ca2+ peaks after thapsigargin, thrombin- or lysophosphatidic acid-mediated stimulation (17,19). Chronic lithium treatment attenuates intracellular calcium mobilization in B lymphoblast cell lines (20). In this context, it has been hypothesized that elevated intracellular Ca2+ levels when associated with mitochondrial dysfunction and subsequent decreased ATP production may reduce the ability of neuronal cells to appropriately respond to temporary peaks in stressful stimuli such as increased glutamate release during emotional distress (21).

Oxidative stress refers to the cytotoxic consequences of excessive generation of ROS – superoxide anion (O2-·), hydrogen peroxide (H2O2), and hydroxyl radical (OH·) (22). Under normal circumstances, ROS are eliminated by enzymatic (i.e. superoxide dismutase, SOD; catalase, CAT; glutathione peroxi-

dase, GPx) and non-enzymatic antioxidant defences (i.e. vitamins; glutathione, GSH). When these antioxidant systems are outweighed by extreme levels of ROS, there is a higher potential for damage to DNA, lipids (i.e. cell and organelle membranes) and proteins (i.e. receptors, transcription factors and enzymes) (23). A number of studies reported increased oxidative damage in both peripheral blood and postmortem brain of BD patients (6,24). For instance, one study found a remarkably high frequency of DNA damage (as measured by DNA fragmentation using the comet assay) in peripheral blood of BD subjects relative to controls and that the frequency of DNA damage correlated with the severity of manic and depressive symptoms (25). Consistent with this finding, a postmortem study found that DNA fragmentation is also increased in non-GABAergic neurons in the anterior cingulate cortex in individuals with BD (26). Another consistent finding that has been replicated by several laboratories is increased lipid peroxidation (lipid damage) in BD. Increased levels of 4-hydroxynonenal, a product of lipid peroxidation, were found in the anterior cingulate cortex of subjects with BD and SZ (27). Several studies have reported increased levels of thiobarbituric acid reactive substances (TBARS) in both plasma (28) and serum samples (29-33). Notably, treatment with lithium and valproate at therapeutically relevant concentrations significantly inhibited glutamate-induced increased intracellular Ca+2, lipid peroxidation, protein oxidation, DNA fragmentation, and cell death in primary cultured rat cerebral cortical cells (34,35). In addition, both of these mood stabilizers can also enhance mitochondrial function and protect against mitochondrially-mediated toxicity in SH-SY5Y neuron cell culture (36). Animal models using increased oxidative stress induced by amphetamine exposure found that lithium and valproate reversed and prevented amphetamine-induced TBARS formation in vivo (37). Consistent with this finding, another rat study showed that lithium and valproate were able to modulate the oxidative balance and prevent cerebral DNA damage (38).

Studies looking at antioxidant activity profile in BD patients have been less consistent. For instance, there are reports of increased SOD and CAT activities (31), unaltered SOD and decreased CAT activity (32,39), an imbalance between increased SOD and decreased CAT activities (29), increased GPx activity (29), and also decreased GPx activity (32) in the peripheral blood of BD subjects. A postmortem study showed decreased total GSH levels in prefrontal cortex of patients with BD, SZ and major depression (40). Furthermore, a case study that investigated the oxidative stress profile in two monozygotic twins during a manic episode showed that bipolar twins had higher TBARS, SOD and

DNA damage, and lower CAT. TBARS and SOD were normalized after mood stabilization, whereas CAT and DNA damage remained unaltered after 6 weeks of treatment (41). A recent meta-analysis confirmed that a consistent finding in BD patients is the presence of increased TBARS across all mood states (42). As far as the potential use of antioxidant agents, there are some encouraging data suggesting that N-acetyl cysteine (NAC), a precursor of glutathione, may be a useful adjunctive treatment in reducing oxidative stress, and improving clinical symptoms, quality of life, and functioning in individuals with BD (43, 44).

In summary, there is converging evidence from animal, clinical, and preclinical studies suggesting that BD is associated with altered Ca2+ signaling and gene expression, increased oxidative stress and DNA damage, and mitochondrial dysfunction. Studies in vitro and in vivo support that both lithium and valproate can stabilize mitochondrial function and prevent oxidative stress. Perhaps more importantly, some initial clinical trials support the use of antioxidants, such as NAC, as adjunctive in the treatment of BD.

Brain Imaging and Energy Metabolism in BD

Positron Emission Tomography (PET)

Brain metabolism can be measured by PET technique using a radiolabelled glucose analogue, [18F]-fluorodeoxyglucose (FDG). When FDG is taken up into the cell, it is metabolized by phosphorylation to FDG-6 phosphate and accumulates in the cell, providing an indirect measurement of the local glucose metabolism.

An early study with euthymic and drug-free BD patients and electrical stimulation showed a reduction of FDG uptake in frontal cortex, occipital cortex and basal ganglia compared to healthy controls (HC) (45). Using the subgenual region as region-of-interest (ROI), a population of depressive BD patients presented a reduction of the glucose metabolism in that area compared to HC (46). In the same sample, manic patients (n=4) revealed higher global FDG uptake. Subsequently, this finding has been independently replicated in a sample of depressed BD patients (47). In the amygdala no differences in glucose metabolism between depressive BD, HC and MDD patients were observed. However, individuals that were off-medication presented higher FDG uptake, which suggests that treatment may normalize over activity in the amygdala (48). A study with FDG and cerebral blood flow (CBF) in BD type I and type II patients showed a positive correlation between glucose metabolism and blood flow, but such correlation was less robust in comparison to HC. Also there was a negative correlation between glucose metabolism and CBF in the left pregenual ACC, indicating a local uncoupling flow-metabolism disrup-

tion that might suggest vasomotor or mitochondrial dysfunction (49). A recent FDG-PET study examined a population of depressed and euthymic women with BD type I, as compared to MDD and HC. This study showed a decrease of glucose metabolism in frontal gyri, right cingulate, inferior parietal cortex and angular gyri in BD patients. However, the euthymic BD patients did not display differences in these brain regions (50), which suggested that some metabolic changes may be state-related depending on the brain area. Another study found a reduction in bilateral dorsolateral prefrontal cortex (DLPFC) and left amygdala, and an increase in the left occipital cortex and right temporal cortex in manic subjects with psychosis. However this study used a "clinical diagnosis" rather than the DSM classification for the selection of the sample (51). Benson and col. reported a FDG study with treatment refractory depressed BD patients. In that study, correlation analysis across different brain regions showed higher metabolic association between the left DLPFC, left inferior parietal cortex, thalamus and insula and other brain regions in the BD sample compared to HC (52). These results are in conflict with previous report of global decrease in metabolism in depressed BD patients in comparison HC in DLPFC, insula, striatum, ACC, PFC and subgenual (53).

In summary, measurements of the brain metabolism with PET reported mostly a decrease in metabolic activity in several brain regions of BD patients in comparison to HC. Even though methodological limitations such as low sample size, heterogeneity of the BD population and lack of control of baseline glucose level may have skewed the results, the consistent finding of low FDG levels in the brain of BD cannot be ignored and suggest that a decrease in energy metabolism may be part of the pathophysiology of BD.

Resting State fMRI

Resting state functional MRI is a technique based on the study of low frequency blood oxygen level-dependent oscillations in the absence of any mental task while the subject rests quietly (54). Although the underlying brain activity revealed by this technique has been debatable, it has been suggested that it would identify brain regions that present functional connectivity across each other (55). To date, only four studies have looked at resting state fMRI technique in BD. In a study that included both manic and depressive BD patients, connectivity between pregenual anterior cingulate (pACC) and amygdala, and between thalamus and pallidostriatum were decreased in BD compared to HC, but were similar to MDD patients (56). Another study of manic and mixed BD I patients showed decreased activity in medial prefrontal cortex (MPFC) and the hippocampus as compared to HC (57). In a heterogeneous BD

population of rapid cyclers, euthymic, mixed, and depressive patients, BD subjects showed decreased connectivity between left ventral prefrontal cortex (VPFC) and left amygdala as well as between left VPFC and dorsofrontal and pariental regions. The same study revealed increases in connectivity between left VPFC and contralateral hemisphere (58). In an euthymic pediatric population of BD patients, resting state fMRI revealed a decrease in the connectivity between left DLPFC and contra-lateral temporal gyrus. Using the superior temporal gyrus (STG) as seed point, BD pediatric patients showed decrease in the connectivity between STG and frontal gyri (superior and middle), and between STG and thalamus. However it was observed an increase in the connectivity between STG and parahippocampal gyrus in the patients in comparison to HC (59).

Overall, the preliminary studies of resting state fMRI suggest that individuals with BD present a decrease in the connectivity between a number of corticolimbic regions associated with mood and cognitive control. Whether such abnormal functional connectivity are part of the pathophysiology of the disease or part of a compensatory mechanism it remains to be determined.

Magnetic Resonance Spectroscopy

Magnetic resonance spectroscopy (MRS) is a non-invasive imaging technique that provides in vivo quantification of a number of metabolites in the brain. Single proton MRS (1H-MRS) enables measurement of glutamate (Glu), glutamine (Gln), N-acetyl-L-aspartate (NAA), choline compounds (Cho), creatine and phosphocreatine (Cre), myoinositol (mI) and lactate. For the purpose of reviewing brain energy metabolism status we will focus on the studies reporting absolute levels of these metabolites in the brain. Results describing differences in ratio of the metabolites (e.g., NAA/Cre, NAA/Cho, etc) will not be reviewed.

In mammals, glutamate is the main excitatory neurotransmitter in the CNS. It is synthetized in glutamatergic neurons and astrocytes (60). Measurements of glutamate (Glu), glutamine (Gln), and the combined Glu-Gln (Glx) provide information about the local glutamate metabolism. Higher or lower glutamate metabolites may indicate abnormalities in the local glutamatergic metabolism. A MRS study with a sample of manic and depressive BD patients found a decrease in Glx levels in the frontal cortex and basal ganglia, while there was no alteration in the thalamus (61). In contrast, another study revealed some brain areas with high levels of Glx such as MVPFC, insular cortex and cortical grey matter (62). BD patients in a manic phase did not display differences in Glu, Gln or Glx levels compared to HC in the ACC, parietal-occipital cortex (63) or OFC (64). However, the ratio Gln/

Glu was reported to be higher in the ACC and the parietal-occipital cortex of manic BD patients (63). To date, only one study examined BD patients during depressive state. Depressive BD patients presented an increase of Glu and Glx but not Gln in the ACC (65). Children with BD showed low Gln but not Glu in the ACC compared to HC (66). In asymptomatic children with one parent presenting BD, the Glu and Glx levels were compared to controls in the cerebellar vermis (67). Different treatments for BD have been tested to reveal changes in the glutamate levels in the brain. Pharmacotherapy with riluzole (68) and lithium (69) did not change the Gln/Glu and Glu and Gln levels levels in the ACC after treatment. However, lithium decreased Glx levels in grey matter of BD patients in comparison to valproate (70). The mood-stabilizer lamotrigine showed to increase Gln in the ACC and the MPFC of depressed BD patients (65). Treatment with quetiapine did not change Glx levels in the medial frontal cortex of rapid cyclic BD patients (71). In a sample of depressed BD patients, treament with cytidine decreased Glu/Gln levels in the ACC in comparison to placebo (72).

Glutamate and glutamine levels as biomarkers for local glutamatergic function have shown higher activity in some cortical regions associated with mood and cognitive control (for instance, ACC and OFC). Considering that the glutamate-glutamine cycle is closely linked to energetic metabolism, these results further support that changes in energy metabolism are part of the pathophysiology of BD.

N-acetyl-L-aspartate (NAA) is produced in the mitochondria in the neuronal cell bodies and it is considered a marker of neuronal integrity (73). In heterogeneous samples of manic and depressive subjects, NAA has been found diminished only in the basal ganglia (61). Measurements in thalamus, frontal cortex white matter (61), anterior cingulate (ACC), insular cortex and grey matter (62) did not reveal differences in NAA levels compared to healthy controls (HC). In euthymic BD patients, it has been reported a decrease in NAA levels in hippocampus (74) and an increase in thalamus (75). Proton MRS studies on medial prefrontal cortex (MPFC) (76), frontal cortex (77), and basal ganglia (78) did not find differences in NAA levels in comparison to HC. BD patients, while in manic phase, showed decrease of NAA levels in the orbital frontal cortex (OFC) (64). However, other brain regions such as ACC (63,78), parietal-occipital cortex (63), dorsolateral prefrontal cortex (DLPFC) (79), and frontal cortex (78) did not reveal differences in NAA levels during manic phase in comparison to HC. Only two MRS studies have focused on NAA levels and BD patients during the depressive phase. These studies found no differences in NAA levels in the ACC (65) and frontal cortex (77). Children diagnosed with BD showed lower

levels of NAA in the DLPFC, MPFC (80) and cerebellum (81) compared to HC. No differences were found in NAA levels in the frontal cortex (81,82), posterior cingulate (PC) and occipital cortex (80) in the pediatric population with BD. Two studies in children focused on the ACC presented different results: NAA levels were found unchanged in an earlier study (83). Recently, in another study, it has been shown a decrease of NAA in the ACC of children with BD (80). Pharmacotherapy seems to affect the NAA levels in the brain of BD patients. Lithium (69), riluzole (68), and ethyl-EPA (84) showed to increase NAA concentration in cortical regions after treatment. Lamotrigine (65) produced a reduction in the NAA levels in the ACC of BD patients. Also in a pediatric BD sample, NAA levels in MVPFC but not in VLPFC decreased after treatment with lithium (85). Some pharmacological studies with quetiapine (71), valproate (70), and lithium (70,85) did not show any effect on NAA levels in different cortical regions.

Low NAA levels in the brain have been described in several neurodegenerative disorders (86). Hence a local decrease of NAA in the brain might be indicative of neuronal damage or death. In BD, some brain regions such as hippocampus, OFC and basal ganglia presented a reduction in NAA levels compared to HC, suggesting decreased neuronal integrity. Interestingly, studies in the pediatric population with BD have shown more brain regions with low NAA levels compared to HC, supporting the idea of BD as a neurodevelopmental disorder.

Creatine and phosphocreatine (Cre) are abundant in the brain. Creatine is producted in the liver and kidneys and it is transported into the brain, where it is converted to phosphocreatine in the mitochondria. Phosphocreatine is then transported to the cytosol and donates a phosphate group to ADP to replenish ATP. Hence, Cre levels may be used as a measure of energy utilization in the brain. Two MRS studies investigated Cre levels in heterogeneous groups of BD patients with both depressive and manic states. As with NAA, Cre presented diminished in the basal ganglia (61). Thalamus, frontal cortex white matter (61), anterior cingulate (ACC), insular cortex and grey matter (62) did not show differences in Cre levels in this population compared to HC. In euthymic BD patients, the results of Cre brain levels also followed the NAA levels: in the hippocampus, Cre levels were found diminished (74), whereas in thalamus Cre levels were found increased compared to HC (75). In euthymic BD, Cre levels were compared to HC in the frontal cortex and MPFC (76,77). While in manic BD patients, no changes in Cre levels were found in several brain regions (63, 64, 79), depressive BD patients presented higher Cre levels in the ACC (65) and lower levels in the frontal cortex (77). In a study

with medication-free individuals with BD at various mood states, it has been reported a decrease in Cre levels in the left DLPFC (87). In pediatric BD, Cre levels have been found mostly decreased compared to HC in the ACC, DLPFC, MPFC (80) and cerebellum (81). In the ACC, another study failed to find differences in Cre levels in the same population (66). Also in children and adolescents with BD, Cre levels were normal in the frontal cortex (81), posterior cingulate and occipital cortex (80). Light therapy (88), lithium (61,70,85,89), ethyl-EPA (84), quetiapine (71), and risperidone (83) all failed to show any effect on Cre levels after treatment.

Creatine and phosphocreatine levels as biomarkers for energy consumption in the brain have demonstrated close relationship with NAA in the same brain regions. Although no alterations in Cre levels were found in manic subjects, BD depressive patients showed opposite changes in Cre levels in distinct areas of the PFC (higher Cre in ACC, lower Cre in frontal cortex). Lower Cre levels have been reported in the DLPFC in BD subjects free of medications but this finding needs replication. In early stages of brain development, studies with children diagnosed with BD consistently showed reduced levels of Cre in frontal cortical regions and cerebellum. Together these results suggest that the abnormalities in the Cre metabolism in the frontal regions in BD may be more pronounced during depression and early in the course of the disease.

Lactate is continuously produced during normal metabolism and may serve as a source of energy in conditions of decreased oxidative phosphorilation. Thus, increased levels of lactate may be an indicative of metabolic dysfunction. Lactate levels were higher in the grey matter of depressed or mixed BD type I and II. In the same sample, the analysis of subpopulation of BD type I patients revealed increase levels of lactate compared to HC (62). Lactate levels in ACC were unchanged after treatment with lithium (70). However, after treatment with quetiapine, manic BD patients showed a reduction in the lactate levels in the medial frontal cortex, with patients who responded to the treatment presenting higher decrease (71).

In vivo quantification of lactate in BD brain has been limited. The only study in medication-free patients suggests that increased lactate may be associated with a shift towards anaerobic glycolysis in the grey matter of BD type I.

Results from PET and resting state fMRI suggest that there are local metabolic abnormalities in the brain of BD patients. Most of the cortical brain regions have shown decrease in markers for energy metabolism in BD compared to HC. MRS studies suggest that BD is associated with impaired oxidative phosphorilation and a shift towards anaerobic energy production with subsequent

decrease in total energy production (5). In addition, markers for neuronal integrity have shown regional low levels in BD. Together, these studies suggest that a decrease in energy metabolism associated with mitochondrial dysfunction may impair the connectivity between brain regions (e.g. fronto-limbic circuit) which may, in turn, lead to mood and cognitive impairment observed in BD.

Conclusions

The data reviewed above provide strong support to the hypothesis of mitochondrial dysfunction in BD. Considering that the main physiological roles of mitochondria are related to oxidative phosphorilation, Ca2+ metabolism and apoptosis, it is remarkable that all of these components have been found abnormal in BD. Notably, abnormalities in the mitochondrial ETC, altered Ca2+ signaling, increased oxidative stress and DNA damage have been observed in peripheral blood and in postmortem brain tissue in BD. These findings suggest that, at least in some brain areas of individuals with BD (most notably sub-areas of the prefrontal cortex), there may be a decrease in mitochondrial oxidative phosphorylation, which may, in turn, cause a decrease in ATP production. If this hypothesis is true, then it expected an increase in the production of lactate and a shift towards anaerobic glycolysis. Indeed, increased lactate levels have been reported both in the CSF as well as in the brain (gray matter) of BD subject as measured by MRS in vivo. Studies with FDG-PET have demonstrated that a number of brain regions are associated with a decrease in glucose metabolism. But perhaps more importantly, initial results from resting state fMRI revealed abnormalities in functional connectivity between fronto-limbic areas associated with emotional and cognitive control.

Preliminary data suggest that lithium and, in a much less extent, valproate may help stabilizing mitochondrial function. There is also new data suggesting that the antioxidant agent NAC may be a useful adjunctive option in the treatment of BD. Whether or not the effects on mitochondrial function and oxidative stress are associated with their clinical response it remains to be determined. In the search for biomarkers of disease and treatment response, markers of energy metabolism and oxidative damage are promising candidates to understand the underlying neurobiology of BD. Ultimately, research in this field may help in the development of new treatment agents for this devastating major mental illness.

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References

1. Kupfer DJ. The increasing medical burden in bipolar disorder. JAMA. 2005;293:2528-30.
2. Leboyer M, Kupfer DJ. Bipolar disorder: new perspectives in health care and prevention. J Clin Psychiatry. 2010;71:1689-95.
3. Clay HB, Sillivan S, Konradi C. Mitochondrial dysfunction and pathology in bipolar disorder and schizophrenia. Int J Dev Neurosci. 2011;29:311-24.
4. Kato T, Kato N. Mitochondrial dysfunction in bipolar disorder. Bipolar Disord. 2000;2:180-90.
5. Stork C, Renshaw PF. Mitochondrial dysfunction in bipolar disorder: evidence from magnetic resonance spectroscopy research. Mol Psychiatry. 2005;10:900-19.
6. Berk M, Kapczinski F, Andreazza AC, et al. Pathways underlying neuroprogression in bipolar disorder: focus on inflammation, oxidative stress and neurotrophic factors. Neurosci Biobehav Rev. 2011;35:804-17.
7. Cataldo AM, McPhie DL, Lange NT, et al. Abnormalities in mitochondrial structure in cells from patients with bipolar disorder. Am J Pathol. 2010;177:575-85.
8. Andreazza AC, Shao L, Wang JF, et al. Mitochondrial complex I activity and oxidative damage to mitochondrial proteins in the prefrontal cortex of patients with bipolar disorder. Arch Gen Psychiatry. 2010;67:360-8.
9. Regenold WT, Phatak P, Marano CM, et al. Elevated cerebrospinal fluid lactate concentrations in patients with bipolar disorder and schizophrenia: implications for the mitochondrial dysfunction hypothesis. Biol Psychiatry. 2009;65:489-94.
10. Naydenov AV, MacDonald ML, Ongur D, et al. Differences in lymphocyte electron transport gene expression levels between subjects with bipolar disorder and normal controls in response to glucose deprivation stress. Arch Gen Psychiatry. 2007;64:555-64.
11. Maurer IC, Schippel P, Volz HP. Lithium-induced enhancement of mitochondrial oxidative phosphorylation in human brain tissue. Bipolar Disord. 2009;11:515-22.
12. MacDonald ML, Naydenov A, Chu M, et al. Decrease in creatine kinase messenger RNA expression in the hippocampus and dorsolateral prefrontal cortex in bipolar disorder. Bipolar Disord. 2006;8:255-64.
13. Streck EL, Amboni G, Scaini G, et al. Brain creatine kinase activity in an animal model of mania. Life Sci. 2008;82:424-9.
14. Chinopoulos C, Adam-Vizi V. Calcium, mitochondria and oxidative stress in neuronal pathology. Novel aspects of an enduring theme. FEBS J. 2006;273:433-50.
15. Orrenius S, Zhivotovsky B, Nicotera P. Regulation of cell death: the calcium-apoptosis link. Nat Rev Mol Cell Biol. 2003;4:552-65.
16. Berk M, Bodemer W, van Oudenhove T, et al. Dopamine increases platelet intracellular calcium in bipolar affective disorder and controls. Int Clin Psychopharmacol. 1994;9:291-3.
17. Dubovsky SL, Murphy J, Thomas M, et al. Abnormal intracellular calcium ion concentration in platelets and lymphocytes of bipolar patients. Am J Psychiatry. 1992;149:118-20.
18. Kusumi I, Koyama T, Yamashita I. Thrombin-induced platelet calcium mobilization is enhanced in bipolar disorders. Biol Psychiatry. 1992;32:731-4.
19. Kato T, Ishiwata M, Mori K, et al. Mechanisms of altered Ca2+ signalling in transformed lymphoblastoid cells from patients with bipolar disorder. Int J Neuropsychopharmacol. 2003;6:379-89.
20. Wasserman MJ, Corson TW, Sibony D, et al. Chronic lithium treatment attenuates intracellular calcium mobilization. Neuropsychopharmacology. 2004;29:759-69.
21. Coyle JT, Puttfarcken P. Oxidative stress, glutamate, and neurodegenerative disorders. Science. 1993;262:689-95.
22. Adam-Vizi V, Chinopoulos C. Bioenergetics and the formation of mitochondrial reactive oxygen species. Trends Pharmacol Sci. 2006;27:639-45.

23. Lenaz G, D'Aurelio M, Merlo Pich M, et al. Mitochondrial bioenergetics in aging. Biochim Biophys Acta. 2000;1459:397-404.
24. Steckert AV, Valvassori SS, Moretti M, et al. Role of oxidative stress in the pathophysiology of bipolar disorder. Neurochem Res. 2010;35:1295-301.
25. Andreazza AC, Frey BN, Erdtmann B, et al. DNA damage in bipolar disorder. Psychiatry Res. 2007;153:27-32.
26. Buttner N, Bhattacharyya S, Walsh J, et al. DNA fragmentation is increased in non-GABAergic neurons in bipolar disorder but not in schizophrenia. Schizophr Res. 2007;93:33-41.
27. Wang JF, Shao L, Sun X, et al. Increased oxidative stress in the anterior cingulate cortex of subjects with bipolar disorder and schizophrenia. Bipolar Disord. 2009;11:523-9.
28. Kuloglu M, Ustundag B, Atmaca M, et al. Lipid peroxidation and antioxidant enzyme levels in patients with schizophrenia and bipolar disorder. Cell Biochem Funct. 2002;20:171-5.
29. Andreazza AC, Cassini C, Rosa AR, et al. Serum S100B and antioxidant enzymes in bipolar patients. J Psychiatr Res. 2007;41:523-9.
30. Kapczinski F, Dal-Pizzol F, Teixeira AL, et al. Peripheral biomarkers and illness activity in bipolar disorder. J Psychiatr Res. 2011;45:156-61.
31. Machado-Vieira R, Andreazza AC, Viale CI, et al. Oxidative stress parameters in unmedicated and treated bipolar subjects during initial manic episode: a possible role for lithium antioxidant effects. Neurosci Lett. 2007;421:33-6.
32. Ozcan ME, Gulec M, Ozerol E, et al. Antioxidant enzyme activities and oxidative stress in affective disorders. Int Clin Psychopharmacol. 2004;19:89-95.
33. Savas HA, Gergerlioglu HS, Gurel A, et al. [Increased xanthine oxidase and malondialdehyde levels in euthymic bipolar patients]. Klinik Psikiyatri Dergisi. 2005;8:180-5. [Artículo en turco].
34. Cui J, Shao L, Young LT, et al. Role of glutathione in neuroprotective effects of mood stabilizing drugs lithium and valproate. Neuroscience. 2007;144:1447-53.
35. Shao L, Young LT, Wang JF. Chronic treatment with mood stabilizers lithium and valproate prevents excitotoxicity by inhibiting oxidative stress in rat cerebral cortical cells. Biol Psychiatry. 2005;58:879-84.
36. Bachmann RF, Wang Y, Yuan P, et al. Common effects of lithium and valproate on mitochondrial functions: protection against methamphetamine-induced mitochondrial damage. Int J Neuropsychopharmacol. 2009;12:805-22.
37. Frey BN, Valvassori SS, Reus GZ, et al. Effects of lithium and valproate on amphetamine-induced oxidative stress generation in an animal model of mania. J Psychiatry Neurosci. 2006;31:326-32.
38. Andreazza AC, Kauer-Sant'Anna M, Frey BN, et al. Effects of mood stabilizers on DNA damage in an animal model of mania. J Psychiatry Neurosci. 2008;33:516-24.
39. Ranjekar PK, Hinge A, Hegde MV, et al. Decreased antioxidant enzymes and membrane essential polyunsaturated fatty acids in schizophrenic and bipolar mood disorder patients. Psychiatry Res. 2003;121:109-22.
40. Gawryluk JW, Wang JF, Andreazza AC, et al. Decreased levels of glutathione, the major brain antioxidant, in postmortem prefrontal cortex from patients with psychiatric disorders. Int J Neuropsychopharmacol. 2011;14:123-30.
41. Frey BN, Andreazza AC, Kunz M, et al. Increased oxidative stress and DNA damage in bipolar disorder: a twin-case report. Prog Neuropsychopharmacol Biol Psychiatry. 2007;31:283-5.
42. Andreazza AC, Kauer-Sant'anna M, Frey BN, et al. Oxidative stress markers in bipolar disorder: a meta-analysis. J Affect Disord. 2008;111:135-44.
43. Berk M, Copolov DL, Dean O, et al. N-acetyl cysteine for depressive symptoms in bipolar disorder--a double-blind randomized placebo-controlled trial. Biol Psychiatry. 2008;64:468-75.
44. Dean O, Giorlando F, Berk M. N-acetylcysteine in psychiatry: current therapeutic evidence and potential mechanisms of action. J Psychiatry Neurosci. 2011;36:78-86.

45. Buchsbaum MS, Wu J, DeLisi LE, et al. Frontal cortex and basal ganglia metabolic rates assessed by positron emission tomography with [18F]2-deoxyglucose in affective illness. J Affect Disord. 1986;10:137-52.
46. Drevets WC, Price JL, Simpson JR, Jr., et al. Subgenual prefrontal cortex abnormalities in mood disorders. Nature. 1997;386:824-7.
47. Kegeles LS, Malone KM, Slifstein M, et al. Response of cortical metabolic deficits to serotonergic challenge in familial mood disorders. Am J Psychiatry. 2003;160:76-82.
48. Drevets WC, Price JL, Bardgett ME, et al. Glucose metabolism in the amygdala in depression: relationship to diagnostic subtype and plasma cortisol levels. Pharmacol, Biochem Behav. 2002;71:431-47.
49. Dunn RT, Willis MW, Benson BE et al. Preliminary findings of uncoupling of flow and metabolism in unipolar compared with bipolar affective illness and normal controls. Psychiatry Res. 2005;140:181-98.
50. Hosokawa T, Momose T, Kasai K. Brain glucose metabolism difference between bipolar and unipolar mood disorders in depressed and euthymic states. Prog Neuropsychopharmacol Biol Psychiatry. 2009;33:243-50.
51. al-Mousawi AH, Evans N, Ebmeier KP, et al. Limbic dysfunction in schizophrenia and mania. A study using 18F-labelled fluorodeoxyglucose and positron emission tomography. The British journal of psychiatry : the journal of mental science. 1996;169:509-16.
52. Benson BE, Willis MW, Ketter TA, et al. Interregional cerebral metabolic associativity during a continuous performance task (Part II) : differential alterations in bipolar and unipolar disorders. Psychiatry Res. 2008;164:30-47.
53. Brooks JO 3rd, Wang PW, Bonner JC, et al. Decreased prefrontal, anterior cingulate, insula, and ventral striatal metabolism in medication-free depressed outpatients with bipolar disorder. J Psychiatr Res. 2009;43:181-8.
54. Raichle ME, MacLeod AM, Snyder AZ, et al. A default mode of brain function. Proc Natl Acad Sci U S A. 2001;98:676-82.
55. Fox MD, Raichle ME. Spontaneous fluctuations in brain activity observed with functional magnetic resonance imaging. Nat Rev Neurosci. 2007;8:700-11.
56. Anand A, Li Y, Wang Y, et al. Resting state corticolimbic connectivity abnormalities in unmedicated bipolar disorder and unipolar depression. Psychiatry Res. 2009;171:189-98.
57. Ongur D, Lundy M, Greenhouse I, et al. Default mode network abnormalities in bipolar disorder and schizophrenia. Psychiatry Res. 2010;183:59-68.
58. Chepenik LG, Raffo M, Hampson M, et al. Functional connectivity between ventral prefrontal cortex and amygdala at low frequency in the resting state in bipolar disorder. Psychiatry Res. 2010;182:207-10.
59. Dickstein DP, Gorrostieta C, Ombao H, et al. Fronto-temporal spontaneous resting state functional connectivity in pediatric bipolar disorder. Biol Psychiatry. 2010;68:839-46.
60. Erecinska M, Troeger MB, Wilson DF, et al. The role of glial cells in regulation of neurotransmitter amino acids in the external environment. II. Mechanism of aspartate transport. Brain research. 1986;369:203-14.
61. Port JD, Unal SS, Mrazek DA, et al. Metabolic alterations in medication-free patients with bipolar disorder: a 3T CSF-corrected magnetic resonance spectroscopic imaging study. Psychiatry Res. 2008;162:113-21.
62. Dager SR, Friedman SD, Parow A, et al. Brain metabolic alterations in medication-free patients with bipolar disorder. Arch Gen Psychiatry. 2004;61:450-8.
63. Ongur D, Jensen JE, Prescot AP, et al. Abnormal glutamatergic neurotransmission and neuronal-glial interactions in acute mania. Biol Psychiatry. 2008;64:718-26.
64. Cecil KM, DelBello MP, Morey R, et al. Frontal lobe differences in bipolar disorder as determined by proton MR spectroscopy. Bipolar Disord. 2002;4(6):357-65.
65. Frye MA, Watzl J, Banakar S, et al. Increased anterior cingulate/medial

prefrontal cortical glutamate and creatine in bipolar depression. Neuropsychopharmacology. 2007;32:2490-9.
66. Moore CM, Frazier JA, Glod CA, et al. Glutamine and glutamate levels in children and adolescents with bipolar disorder: a 4.0-T proton magnetic resonance spectroscopy study of the anterior cingulate cortex. J Am Acad Child Adolesc Psychiatry. 2007;46:524-34.
67. Singh MK, Spielman D, Libby A, et al. Neurochemical deficits in the cerebellar vermis in child offspring of parents with bipolar disorder. Bipolar Disord. 2011;13:189-97.
68. Brennan BP, Hudson JI, Jensen JE, et al. Rapid enhancement of glutamatergic neurotransmission in bipolar depression following treatment with riluzole. Neuropsychopharmacology. 2010;35:834-46.
69. Forester BP, Finn CT, Berlow YA, et al. Brain lithium, N-acetyl aspartate and myo-inositol levels in older adults with bipolar disorder treated with lithium: a lithium-7 and proton magnetic resonance spectroscopy study. Bipolar Disord. 2008;10:691-700.
70. Friedman SD, Dager SR, Parow A, et al. Lithium and valproic acid treatment effects on brain chemistry in bipolar disorder. Biol Psychiatry. 2004;56:340-8.
71. Kim DJ, Lyoo IK, Yoon SJ, et al. Clinical response of quetiapine in rapid cycling manic bipolar patients and lactate level changes in proton magnetic resonance spectroscopy. Prog Neuropsychopharmacol Biol Psychiatry. 2007;31:1182-8.
72. Yoon SJ, Lyoo IK, Haws C, et al. Decreased glutamate/glutamine levels may mediate cytidine's efficacy in treating bipolar depression: a longitudinal proton magnetic resonance spectroscopy study. Neuropsychopharmacology. 2009;34:1810-8.
73. Urenjak J, Williams SR, Gadian DG, et al. Proton nuclear magnetic resonance spectroscopy unambiguously identifies different neural cell types. J Neurosci. 1993;13:981-9.
74. Deicken RF, Pegues MP, Anzalone S, et al. Lower concentration of hippocampal N-acetylaspartate in familial bipolar I disorder. Am J Psychiatry. 2003;160:873-82.
75. Deicken RF, Eliaz Y, Feiwell R, et al. Increased thalamic N-acetylaspartate in male patients with familial bipolar I disorder. Psychiatry Res. 2001;106:35-45.
76. Hajek T, Bernier D, Slaney C, et al. A comparison of affected and unaffected relatives of patients with bipolar disorder using proton magnetic resonance spectroscopy. J Psychiatry Neurosci. 2008;33:531-40.
77. Hamakawa H, Kato T, Shioiri T, et al. Quantitative proton magnetic resonance spectroscopy of the bilateral frontal lobes in patients with bipolar disorder. Psychological medicine. 1999;29:639-44.
78. Malhi GS, Ivanovski B, Wen W, et al. Measuring mania metabolites: a longitudinal proton spectroscopy study of hypomania. Act Psychiatr Scand Suppl. 2007:57-66.
79. Frey BN, Folgierini M, Nicoletti M, et al. A proton magnetic resonance spectroscopy investigation of the dorsolateral prefrontal cortex in acute mania. Human psychopharmacol. 2005;20:133-9.
80. Caetano SC, Olvera RL, Hatch JP, et al. Lower N-acetyl-aspartate levels in prefrontal cortices in pediatric bipolar disorder: a (1)H magnetic resonance spectroscopy study. J Am Acad Child Adolesc Psychiatry. 2011;50:85-94.
81. Cecil KM, DelBello MP, Sellars MC, et al. Proton magnetic resonance spectroscopy of the frontal lobe and cerebellar vermis in children with a mood disorder and a familial risk for bipolar disorders. J Child Adolesc Psychopharmacol. 2003;13:545-55.
82. Castillo M, Kwock L, Courvoisie H, et al. Proton MR spectroscopy in children with bipolar affective disorder: preliminary observations. AJNR Am J Neuroradiol. 2000;21:832-8.
83. Moore CM, Biederman J, Wozniak J, et al. Mania, glutamate/glutamine and risperidone in pediatric bipolar disorder: a proton magnetic resonance spectroscopy study of the anterior cingulate cortex. J Affect Disord. 2007;99:19-25.
84. Frangou S, Lewis M, Wollard J, et al. Preliminary in vivo evidence of increased N-acetyl-aspartate following eico-

sapentanoic acid treatment in patients with bipolar disorder. J Psychopharmacol. 2007;21:435-9.
85. Patel NC, DelBello MP, Cecil KM, et al. Temporal change in N-acetyl-aspartate concentrations in adolescents with bipolar depression treated with lithium. J Child Adolesc Psychopharmacol. 2008;18:132-9.
86. Rigotti DJ, Inglese M, Gonen O. Whole-brain N-acetylaspartate as a surrogate marker of neuronal damage in diffuse neurologic disorders. AJNR Am J Neuroradiol. 2007;28:1843-9.
87. Frey BN, Stanley JA, Nery FG, et al. Abnormal cellular energy and phospholipid metabolism in the left dorsolateral prefrontal cortex of medication-free individuals with bipolar disorder: an in vivo 1H MRS study. Bipolar Disord. 2007;9(Suppl 1):119-27.
88. Benedetti F, Calabrese G, Bernasconi A, et al. Spectroscopic correlates of antidepressant response to sleep deprivation and light therapy: a 3.0 Tesla study of bipolar depression. Psychiatry Res. 2009;173:238-42.
89. Deicken RF, Fein G, Weiner MW. Abnormal frontal lobe phosphorous metabolism in bipolar disorder. Am J Psychiatry. 1995;152:915-8.

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