DORSAL ANTERIOR CINGULATE LACTATE AND GLUTATHIONE LEVELS IN EUTHYMIC BIPOLAR I DISORDER: 1H-MRS STUDY

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Abstract

Objective: Oxidative stress and mitochondrial dysfunction are two closely integrated processes implicated in the physiopathology of bipolar disorder (BD). Advanced $^1$H-MRS techniques enable the measurement of levels of lactate (Lac), the main marker of mitochondrial dysfunction, and glutathione (GSH), the predominant brain antioxidant. The objective of this study was to measure brain Lac and GSH levels in BD and healthy controls (HC).

Methods: Eighty-eight individuals (50 BD and 38 HC) underwent 3T $^1$H-MRS in the dorsal anterior cingulate cortex (dACC) (2x2x4.5 cm$^3$) using a 2-D JPRESS sequence. Lac and GSH were quantified using the ProFit software program. Results: BD patients had higher dACC Lac levels compared to controls. GSH levels did not differ between euthymic BD and controls. There was a positive correlation between Lac and GSH levels specific to BD. No influence of medications on metabolites was observed. Conclusion: This is the most extensive MRS study of Lac and GSH in BD to date and results indicated that euthymic BD patients had higher levels of Lac, which might be an indication of altered mitochondrial function. Moreover, Lac levels correlated with GSH levels, indicating a compensatory mechanism regardless of BD diagnosis.

Keywords: glutathione, lactate, bipolar disorder, mitochondrial disease, oxidative stress

Significant outcomes

1- Higher dorsal anterior cingulate Lac levels in euthymic BD compared to controls;

2- No difference in dACC GSH levels between BD and controls;

3- Positive correlation between Lac and GSH levels in BD.

Limitations

1- The $^1$H-MRS voxel did not include only dACC, but also dorsomedial prefrontal cortex (DMPFC) and surrounding white matter.

2- Excluding Lac levels with CRLB=999% could cause the exclusion of subjects with extremely low levels of Lac, but with high chance of error in the measurement.

3- We did not use any scale to measure alcohol consumption.
Background

Multiple neurobiological pathways have been implicated in the physiopathology of bipolar disorder (BD). Oxidative stress (Berk et al., 2011; Soeiro-de-Souza et al., 2013) and mitochondrial dysfunction number amongst these pathways (Stork and Renshaw, 2005). Although these two processes are closely integrated, published reports have generally focused on only one of these abnormalities at a time. Proton magnetic resonance spectroscopy ($^1$H-MRS) is a noninvasive method that allows in vivo detection of metabolic alterations in localized brain areas (voxels). With regard to BD, previous studies based on standard one dimensional $^1$H-MRS protocols report a possible glycolytic shift evidenced by abnormalities in the glutamate to glutamine ratio (Stork and Renshaw, 2005; Soeiro de Souza et al., 2013; 2015). Two-dimensional (2D) J-resolved $^1$H-MRS techniques allow the measurement of small signal metabolites such as lactate (Lac), the main metabolic marker of mitochondrial dysfunction, and glutathione (GSH), the predominant brain antioxidant. Currently, there are no 2D J-resolved $^1$H-MRS studies reporting measures of both Lac and GSH concomitantly in BD. Measuring these two metabolites at the same time in vivo provides a unique opportunity to test the hypotheses of altered redox state and its association with mitochondrial dysfunction in BD.

Mitochondria play a crucial role in ATP production through oxidative phosphorylation, a process carried out by the respiratory chain complexes I, II, III, and V (Orth and Schapira, 2001; Shanske et al., 2001; Chinnery and Schon, 2003). When mitochondrial function is inhibited or insufficient, anaerobic glycolysis is activated, leading to higher production of Lac. Thus, the accumulation of Lac occurs when oxidative phosphorylation is unable to meet energy requirements and the cell is forced to rely on the glycolytic process (Rudkin and Arnold, 1999). In general, mitochondrial dysfunction contributes to neurodegeneration either by apoptosis or generation of reactive oxygen species (ROS). ROS such as hydrogen peroxide, superoxide and hydroxyl radicals are produced as by-products of mitochondrial phosphorylation (Gutteridge and Halliwell, 2000; Cavanagh et al., 2002; Clark et al., 2002; Ferrier and Thompson, 2002). Under these circumstances of elevated levels of Lac and ROS,
the GSH system, as the major brain antioxidant, has a fundamental role.

The GSH system is especially important for cellular defense against ROS, as GSH is the major antioxidant in the brain. This system comprises the enzymes that synthesize GSH within cells, as well as dedicated enzymes that use GSH as the means to exert antioxidant effects (Dringen, 2000). GSH reacts directly with radicals in nonenzymatic reactions and is the electron donor in the reduction of peroxides catalyzed by GSH peroxidase. Astrocytes appear to contain higher GSH levels than neurons both in vivo and in culture (Dringen, 2000). There are few reports about abnormalities of the GSH system in BD regarding altered enzymes that use GSH as a co-factor (GSH reductase, GSH S-transferase, GSH peroxidase), but the interpretation of findings is limited by the heterogeneous methodologies used (Brown et al., 2014). Studies conducted on peripheral blood cells have shown that BD is associated with increased levels of GSH reductase and GSH S-transferase in the late stage of the illness (Andreazza et al., 2009). Moreover, low GSH S-transferase levels have been reported in post-mortem prefrontal cortex from patients with BD, major depressive disorder and schizophrenia (Gawryluk et al., 2011). Additionally, elevated GSH peroxidase in peripheral blood has been reported in BD depressive episodes (Andreazza et al., 2007; de Sousa et al., 2014) but not in mania or euthymia (Abdalla et al., 1986; Kuloglu et al., 2002; Andreazza et al., 2009; Raffa et al., 2012).

Previous 1H-MRS studies investigating GSH are scarce in BD because standard techniques are not sufficiently sensitive to detect metabolites present in low concentrations, such as GSH. Our review identified five previous MRS studies on GSH in BD, all of which included a mixed sample of BD patients (type I, II or NOS) in mania, depression or euthymia (Chitty et al., 2013; Lagopoulos et al., 2013; Chitty et al., 2014; Godlewska et al., 2014; Chitty et al., 2015). All of these studies reported no differences in GSH levels between BD and controls in anterior cingulate (ACC) (Chitty et al., 2013; Lagopoulos et al., 2013; Chitty et al., 2014), prefrontal / occipital voxels (Godlewska et al., 2014) or hippocampus (Chitty et al., 2015). With the objective of confirming an absence of differences in GSH between BD patients and
HC we studied a large and homogeneous sample of euthymic BD type I patients. Furthermore, the 2D-J resolved-PRESS $^1$H-MRS technique was employed for its greater sensitivity in detecting small GSH signals compared to the conventional $^1$H-MRS technique used in previous studies.

Previous evidence on oxidative stress in BD has shown alterations in antioxidant enzymes and lipid peroxidation in different states and stages of BD (Berk et al., 2011). Superoxide dismutase and catalase activity have been reported to be altered in BD mood episodes (Andreazza et al., 2007; Machado-Vieira et al., 2007). Evidence for oxidative stress in BD has been found in the form of lipid peroxidation and reduced Na$^+$-K$^+$-ATPase activity, alterations which can be counteracted by lithium treatment (Banerjee et al., 2012). Moreover, decreased plasma levels of total glutathione and GSH, together with lower catalase expression, increased protein carbonyls, 4-HNE, and 3-NT were found in BD patients (Raffa et al., 2012; Andreazza et al., 2013). Mitochondrial abnormalities in BD patients displayed decreased attachment of hexokinase 1 to outer mitochondrial membrane and decreased Complex I levels (Andreazza et al., 2013). Moreover, there is some evidence indicating that the lifetime number of manic episodes increases oxidative damage to guanosine in BD, where our group previously reported an association between elevated levels of 8-OHdG and number of manic episodes (Soeiro-de-Souza et al., 2013).

Elevated Lac has been considered a marker of mitochondrial dysfunction in BD (Stork and Renshaw, 2005). Brain Lac, a metabolic product of glycolysis, plays an integral role in neuronal energy metabolism (Schurr, 2006). Lac exists in the healthy brain at low basal concentrations, and elevations can indicate transient changes in physiological state (Dager et al., 1999) or neural activation (Frahm et al., 1996). Lac is a metabolite that is hard to measure due to its low concentration as well as to difficulties distinguishing the Lac signal from that of overlapping lipids and macromolecules, where specific $^1$H-MRS- methods are required to reliably detect Lac (Rudkin and Arnold, 1999). The four previous $^1$H-MRS studies on Lac comparing BD patients to controls have reported increased levels in patients with BD (Dager
et al., 2004; Brady et al., 2012; Chu et al., 2013; Xu et al., 2013) but none have exclusively investigated BD type I euthymic patients. These studies have included a mixture of BD subjects in different mood episodes, where this might be problematic when studying Lac in BD, as far as anti-oxidative enzymes have been reported to be altered during mood episodes (Andreazza et al., 2007; Machado-Vieira et al., 2007). Moreover, the majority of previous Lac $^1$H-MRS studies used a short echo-time (TE), which can obscure Lac detection due to an important overlap with lipids’ signal in this region (Rudkin and Arnold, 1999).

Aims of the Study
The aim of this study was to measure simultaneously dorsal anterior cingulate cortex (dACC) levels of Lac and GSH in euthymic BD type I and healthy controls (HC), using in vivo 2D $^1$H-MRS. We hypothesized that BD patients present higher levels of Lac and lower levels of GSH based on the assumption of increased redox state and mitochondrial dysfunction in BD.

Methods
Eighty-eight subjects were included in this study. Of these, 50 (31 F, 18-45 years old) were euthymic BD I subjects and 38 (15 F, 18-45 years old) were healthy controls (HC). Diagnoses were made by trained psychiatrists based on the Structured Clinical Interview (SCID-I/P) (First et al., 1996) for DSM-IV TR (DSM-IV, 2000). The subjects had been on stable medication regimens for at least 2 months prior to the scanning session. Subjects with neurological disorders or medical disorders, head trauma, or current/past (3 months) substance abuse, as well as those who had been treated with electroconvulsive therapy in the last six months were excluded. Moreover subjects referring heavy episodic drinking (consuming five or more standard drinks (male), or four or more drinks (female), over a 2-hour period) (Moreira et al., 2009) over the past 3 months were excluded. The Young Mania Rating Scale (YMRS) (Young et al., 1978) and the Hamilton Depression Rating Scale (HDRS-21) (Hamilton, 1960) were used to assess residual sub-threshold depressive and manic symptoms. Euthymia was defined as <7 YMRS and < 7 HDRS. The patients also fulfilled the DSM-IV criteria for remission.
All HC had no current or past history of psychiatric disorders according to the evaluation conducted by trained psychiatrists using the Mini International Neuropsychiatric Interview (MINI) (Sheehan et al., 1998). In addition, HC subjects had no family history of mood or psychotic disorders among first-degree relatives based on a semi-structured interview.

The research ethics committee CEP CAPPesq from the University of Sao Paulo approved the study. Written informed consent was obtained from all study participants.

**Image Acquisition**

All MRI exams were performed on a Philips 3T Achieva scanner (Philips Healthcare, Best, The Netherlands) using an eight-channel head coil. Spectroscopy measurements were performed using the maximum echo sampled JPRESS sequence proposed by Schulte et al. (Schulte et al., 2006). The JPRESS sequence is based on the conventional PRESS spin-echo technique used for selection of a single voxel. By varying the echo time of the acquisition, the J coupling evolution is encoded in an additional dimension. This technique is therefore also known as two-dimensional spectroscopy, whereby the signal is measured as a function of chemical shift expressed by the Larmor frequency (as in conventional one-dimensional spectroscopy) but also as a function of the coupling constant J in Hz. With the information of the coupling constant J, it is possible to resolve the signals from overlapping multiplets, such as Lac and GSH. In this study, the JPRESS sequence was used to evaluate a voxel of 20 mm (L-R) x 20 mm (I-S) x 45 mm (A-P) (total voxel size 18 cm$^3$) in the dACC region, as shown in figure 1. The minimum echo time (TE) used was 31 ms, and TE was incremented in 100 steps of 2 ms each. For each time increment $\Delta$TE, the maximum-echo sampling started the acquisition $\Delta$TE/2 earlier with respect to the echo top (Schulte et al., 2006). The repetition time (TR) was 1600 ms, and 8 averages were acquired for each TE step. One non-water suppressed spectrum was also acquired at each TE. The number of points per spectrum was 1024, and the spectral bandwidth was 2000 Hz. An automatic second order $B_0$ shimming routine was used and water suppression was achieved by VAPOR (Tkác et al., 1999).
Spectroscopy acquisition took 24 minutes, and the total exam duration, including volumetric imaging and voxel planning, was about 45 minutes. Metabolite quantification was obtained using ProFit (PRiOr knowledge FITting) version 2.0 running on Matlab R2011b (Fuchs et al., 2013). The first version of ProFit was developed by Shulte et al. (Schulte and Boesiger, 2006) to fit 2D JPRESS data by extending LCMModel (Provencher, 1993) principles to 2D data sets. In ProFit, as in the LCMModel approach, the prior knowledge comes from a known metabolite basis set (experimentally acquired or calculated) used in the fitting process, and the VARPRO approach (van der Veen et al., 1988) is used to separate the optimization of non-linear and linear parameters for faster convergence. Fuchs et al. improved the quantification program (ProFit version 2.0) by introducing an experimentally-acquired 2D macromolecular baseline into the fitting model and allowing for a more accurate and precise fit by accounting for the actual line shape and additional baseline distortions by self-deconvolution and spline modeling approaches (Fuchs et al., 2013).

The metabolite basis set used by ProFit includes spectra from a total of 18 brain metabolites including the metabolite of interest in this study: Lac and GSH. Basis set metabolite spectra were calculated with the GAMMA library (smith et al., 1994) using the chemical shift and J-coupling values from the literature (Fan, 1996; Govindaraju et al., 2000). Quantitative results in ProFit are given in the form of ratios to Cr signal (met/Cr). These ratios are already corrected for T2 relaxation effects, since ProFit automatically calculates T2 relaxation times for each metabolite from the signal obtained at the different TEs. “Pseudo” absolute metabolite values [met] were obtained by assuming a white matter (WM) Cr concentration of 4.83 mM (mmol/L) and a grey matter (GM) Cr concentration of 9.59 mM, as expressed in the equation below. These Cr values in mM were calculated from previously reported Cr concentrations in units of mmol/kg (Gasparovic et al., 2006), as used previously (Soeiro de Souza et al., 2015; Zoelch et al., 2015) and expressed in the following equation:

\[
\text{met} = \left( f_{GM} \times 9.59\text{mM} + f_{WM} \times 4.83\text{mM} \right) \times \frac{\text{met}}{\text{Cr}}
\]

where
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\[ f_{\text{GM}} = \frac{\text{GM}\%}{\text{GM}\% + \text{WM}\%} \quad \text{and} \quad f_{\text{WM}} = \frac{\text{WM}\%}{\text{GM}\% + \text{WM}\%} \]

T1 relaxation effects were corrected for, assuming a mono-exponential T1 relaxation with GM T1 of 1.46s and a WM T1 of 1.24s (Mlynárik et al., 2001).

GM % and WM % represent grey and white matter volume percentages, respectively, in the selected MRS voxel, while \( f_{\text{GM}} \) and \( f_{\text{WM}} \) represent the fractions of Cr signal attributable to GM and WM, respectively. To determine the brain tissue composition contained in the MRS voxel of interest, three-dimensional volumetric images were obtained using the 3D-T1-FFE (fast field echo) technique (FA=8°; TE/TR/TI=3.2/7/900 ms) with an isotropic voxel size of 1 mm\(^3\). Briefly, the brain tissue was extracted using the brain extraction tool (BET), and segmentation into WM, GM, and CSF was achieved using the automated brain segmentation tool FAST (Zhang et al., 2001). Both tools are part of the FSL suite (http://www.fmrib.ox.ac.uk/fsl). Finally, the MRS voxel was overlaid on the segmented image using a Python-based script developed in-house and percentages of WM, GM and CSF were calculated for each voxel. The ProFit program also provides a Cramér-Rao lower bound (CRLB) (Cavassila et al., 2001), a measure of the quality of the metabolite quantification, for each metabolite. CRLB were noted for each metabolite. Excluding spectra with Lac CRLBs above a specific threshold (30%) is a common practice in the literature on Lac. However, it was recently shown that to do so might prevent the observation of real differences between groups (Kreis, 2016), so we decided not to set a CRLB cutoff point in this study to avoid a misinterpretation of our Lac results. Only data with a CRLB of 999% were discarded from the analysis, since this specific CRLB output denotes that the program failed to calculate a reliable CRLB.

**Statistical Analysis**

The sample was first tested for homogeneity. Categorical variables were analyzed using \( \chi^2 \) tests whereas continuous variables were analyzed using \( t \)-tests. Normality was checked using the Kolmogorov-Smirnov test. Significant differences in age and gender were observed in the
sample and to prevent this potential bias from influencing results, age and gender correction was performed in all analyses. Normally distributed variables were compared between the two groups using ANCOVAs in which Lac or GSH were entered as a dependent variable while age, gender, group, CSF (only for Lac) and $f_{GM}$ were entered as covariates. To investigate the influence of medication use on Lac and GSH we performed a ANCOVAs test in which Lac or GSH were entered as a dependent variable while medication type (lithium, atypical antipsychotics or anticonvulsants), age, gender, CSF (only for Lac) and $f_{GM}$ were entered as covariates. To investigate the influence of illness duration or lifetime psychotic symptoms on metabolites we used a ANCOVAs test in which Lac or GSH was entered as dependent variable while illness duration or lifetime psychotic symptoms, age, gender, CSF (only for Lac) and $f_{GM}$ were entered as covariates. Finally, to investigate the correlation between GSH and Lac we performed a regression analysis controlled by age and gender. All statistical analyses were carried out using IBM SPSS version 20.

**Results**

After controlling for age and gender, significant differences were observed between BD patients and HC in voxel content for GM% (BD 52% vs. HC 54%) ($F=5.5$, df=88, $p=0.02$) and CSF% (BD 25% vs. HC 23%) ($F=6.2$, df=88, $p=0.01$), but no differences were observed in WM content (BD 23% vs. HC 23%) ($F=0.4$, df=88, $p=0.48$). Given the observed differences between HC and BD in voxel composition we performed also a more detailed segmentation analysis of the brain tissue contributing to the spectrum, by overlaying the $^1$H-MRS voxel on to structural maps segmented by FreeSurfer software (https://surfer.nmr.mgh.harvard.edu). This volumetric analysis is part of a separate publication including a larger cohort of BD patients (to be submitted). Results of the MRS voxel overlay onto the segmented volumetric images indicate that the cortical structure driving this difference in voxel brain tissue composition was the caudal anterior cingulate, which was reduced in BD compared to HC (HC=16.5±3.5% vs. BD=14.1±3.8%; $p=0.005$). As a result BD subjects had lower GM volume and higher CSF volume within the MRS
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voxel. These differences were compensated for in the statistical analysis by inclusion of f<sub>GM</sub> (as described above in Image acquisition) as a variable in the statistical model. When comparing Lac between groups CSF% was also considered as a covariate, since Lac can be present in CSF. GSH was present and quantifiable for all patients with a mean CRLB of 3.98% (1.37%-5.71%). Group characteristics for the GSH analysis are described in Table 1. There was no statistically significant difference in GSH levels between groups, although the BD group had higher mean levels (f=2.9 df=88 p=0.08) (BD mean 1.32 ± 0.31; HC mean 1.24 ± 0.14).

Regarding the Lac MRS, 16 individuals (3 HC and 13 BD) were excluded for having CRLB of 999%, indicating lipid contamination or other artifacts (Supplement Table 1). Therefore, the sample used for Lac analysis consisted of 37 BD and 35 HC (Table 2). A statistically significant difference in Lac level was observed between the groups; the BD group had higher mean levels (F=5.49 df=72 p=0.02) (BD mean 0.31 ± 0.15; HC mean 0.24 ± 0.09) compared to the HC group (Figure 2). As supplementary material, an analysis was performed considering CRLB<30% (sample size BD=26 x HC=26) whose outcome supported the result of higher Lac in BD (F=5.0 df=52 p=0.02) (Supplement Table 2).

We observed a positive correlation between Lac and GSH levels in BD (B=0.20 t=3.2 p=0.003 CI= 0.07 – 0.33) but not in HC (B=0.17 t=1.64 p=0.11 CI= -0.04 – 0.39) (Figure 3).

When we perform this analysis without correcting for age, gender or fGM we observed the same results: In BD Lac was correlated with GSH (B=0.21 t=3.44 p=0.002 CI= 0.08 – 0.33), while in HC there was no correlation between these two metabolites (B=0.19 t=1.8 p=0.07 CI= -0.01 – 0.41).

Neither illness duration nor the presence of lifetime psychotic symptoms demonstrated to influence any of the metabolites measures (p<0.05).

There was no influence of lithium, atypical antipsychotics or anticonvulsants on Lac or GSH levels.

Discussion
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In the present study, higher dACC Lac levels were found in euthymic BD type I patients compared to HC. No significant differences in GSH were observed, but a positive correlation between Lac and GSH level was detected. Moreover, no influence of medications on metabolite levels was found.

Our results demonstrated increased levels of Lac in the dACC of BD type I patients regardless of medication use. Our results revealed increased Lac in BD regardless of medication use, supporting the hypothesis of mitochondrial dysfunction in BD (Stork and Renshaw, 2005). Mitochondrial dysfunction occurs when oxidative phosphorylation is unable to meet energy requirements and the cell is forced to rely on the glycolytic process, which increases the production of Lac (Moore and Galloway, 2002). Some studies have reported higher Lac concentrations in the CSF of BD patients (Regenold et al., 2009). Furthermore, post-mortem studies have found decreased expression of mitochondrial genes encoding the electron transport chain (Konradi et al., 2004; Sun et al., 2006) and abnormal mitochondrial complex I activity (Andreazza et al., 2010) in brain of BD individuals. Moreover, the presented data is reinforced by other MRS studies reporting lower intracellular pH (Kato et al., 1992; 1993), decreased N-acetyl aspartate (Cecil et al., 2002; Chang et al., 2003) and higher Glx (Yüksel and Ongur, 2010; Soeiro de Souza et al., 2013). 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 (Stork and Renshaw, 2005).

Our data evidencing increased Lac in BD is in agreement with the majority of previous Lac ¹H-MRS studies involving mania, depression and euthymia (Dager et al., 2004; Chu et al., 2013; Xu et al., 2013). Only one study, by Brady et al 2012 reported lower Lac levels in BD patients (n=7) compared to HC. Brady et al. reported that Lac levels in mania (n=7) were increased compared to HC in the parietal occipital cortex and ACC. However, when these same patients were in euthymia after treatment, their Lac levels were lower (n=7) than those of HC (n=6) (Brady et al., 2012). Chu et al. investigated Lac levels (4T scanner) in a sample of 21 BD patients in euthymia and mixed episode compared to 10 HC. The group reported
increased Lac levels in BD patients, symptomatic or otherwise (Chu et al., 2013). The other two studies comparing Lac levels in BD patients with HC reported increased levels of Lac in the ACC of BD patients during mania, depression and mixed state (Dager et al., 2004; Xu et al., 2013). Three out of the four Lac MRS studies described CRLB levels and used a CRLB cutoff for inclusion of subjects in the study. Brady et al. included individuals with CRLB<26% (Brady et al., 2012), while Xu et al. and Chu et al. included those with CRLB ≤30% (Chu et al., 2013; Xu et al., 2013). A known problem with measuring Lac is systematic over-estimation when using linear combination fitting algorithms to estimate concentrations (Kreis, 2004). Excluding spectra with Lac CRLBs above a specific threshold (30%) is a common practice in the literature on Lac since CRLBs are linked to chemical estimation reliability. However, due to the difficulty discriminating the Lac signal from background noise, the meaning of the CRLBs produced by LCMModel for this metabolite is less clear. Kreis et al. reported that using CRLB threshold as an exclusion criterion can lead to false conclusions, and suggested that quality checks should be based on metabolites present at higher concentrations (Kreis and Kyathanally, 2015; Kreis, 2016). Therefore, we decided not to set a CRLB cutoff point in this study to avoid misinterpretation of our Lac data, and by doing so the mean CRLB of Lac in this study was 30.8%. The Supplementary material (Supplement Table 2) demonstrates that inclusion of only individuals with CRLB<30% would have yielded a smaller sample size (n BD=26; n HC=26) with similar results.

Our GSH data is in agreement with the five previous MRS studies on GSH in BD (Chitty et al., 2013; Lagopoulos et al., 2013; Chitty et al., 2014; Godlewska et al., 2014; Chitty et al., 2015). All studies reported no differences in GSH levels between BD and controls within different voxels, but included BD type I, II and BD spectrum during all mood episodes. Two of these studies reported a negative association between alcohol and tobacco use with GSH levels in the ACC, that was specific to BD patients (Chitty et al., 2013; 2014). A longitudinal study reported that elevated GSH levels in the hippocampus were associated with lower alcohol consumption and frequency of tobacco use (Chitty et al., 2015). Based on our data, from a sample that included exclusively euthymic patients, taken together with previous GSH
\(^1\)H-MRS studies, it can be concluded that the levels of GSH measured by \(^1\)H-MRS do not differ between BD patients and HC, regardless of mood state. In BD patients we found a correlation between Lac and GSH, where higher Lac was associated with higher GSH levels. We hypothesize that the positive correlation found between GSH and Lac could be related to the presence of a physiological compensatory mechanism, in which there is an increase in GSH levels as Lac levels increase. Similar results have been observed in early stage schizophrenia and post-traumatic stress disorder (PTSD) where higher GSH was noted in subjects with greater oxidative stress (Michels et al., 2014; Wijtenburg et al., 2015). We hypothesize that both BD and HC groups had different pattern of correlation between Lac and GSH because the patients were medicated and stable, allowing a compensatory response of GSH to increased mitochondrial dysfunction. Therefore, we speculate that the association between Lac and GSH is impaired during acute mood episodes.

A limitation to studying euthymic BD type I patients is the difficulty finding subjects without symptoms who are not under medication treatment. Therefore, the use of medications should always be controlled as a cofactor in this type of study. Consequently, we can only state that the findings reported are the result of an interaction among all the factors and probably a metabolic feature of euthymic medicated BD type I patients. Moreover, the present sample of BD patients differed from HC for age and gender, although these variables were controlled for in all analysis. Furthermore, the differences in tissue voxel composition observed between BD patients and HC are probably explained by cortical thinning, previously reported for BD (Lyoo et al., 2006) and major depressive disorder (MDD) (Li M et al., 2014). In congruence with this phenomenon, we found a smaller contribution of the caudal anterior cingulate cortex to the total brain tissue in the MRS voxel among BD patients compared to HC. In order to compensate for this variation the f\(_{GM}\) and CSF\(\%\) were considered in the statistical analysis.

To the best of our knowledge, this investigation had the largest sample size for an MRS study of Lac and GSH in euthymic BD type I patients. Our data showed that BD type I patients had higher levels of Lac in the dACC, which could be an indication of altered mitochondrial function and a glycolytic shift in BD even during euthymia. Moreover, Lac correlated with
GSH levels regardless of BD diagnosis, indicating a physiological association between the anti-oxidative system and mitochondrial dysfunction.

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Figure legend section:

Figure 1- MRS voxel location in the sagittal plane. Size 20 x 20 x 45 mm³

Figure 2- Mean dACC levels of Lactate in BD type I compared to healthy controls

Figure 3- Correlation between dACC levels of Lactate and Glutathione adjusted for age and gender
Figure 1. MRS voxel location in the sagittal plane. Size 20 x 20 x 45 mm$^3$. 
Figure 2. Mean dACC levels of Lactate in BD type I compared to healthy controls

p = 0.02
Figure 3. Correlation between dACC levels of Lactate and Glutathione adjusted for age and gender.