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Comparison of Metabolic Profiling of Cyanidin-3-*O*-galactoside and Extracts from Blueberry in Aged Mice

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ABSTRACT: The metabonomics changes of plasma and brain tissue after dietary supplementation with blueberry extracts (BBE) and cyanidin-3-*O*-galactoside from blueberry (BBM) in aged mice were investigated by ^{1}H NMR technique. The mice received intragastric administration of BBE (200 mg/kg/day), BBM (50 mg/kg/day), and saline water (0.9%) for 6 weeks, respectively, in the BBE, BBM, and control groups. At the end of the experiment, plasma and brain samples were collected for NMR analysis. The results demonstrated that the level of choline in plasma from BBE and BBM groups were obviously elevated relative to the control group, whereas the levels of lactate and phosphocholine in plasma were remarkably reduced. Compared with those in the control group, the levels of choline and GABA in the brain from the BBE group were obviously increased, whereas glutamate and phosphocholine in the BBE group were significantly decreased. The level of taurine in the brain from the BBM group was particularly higher than that in the control group. These results indicated supplementation with BBE or BBM might induce similar changes of endogenous plasma and brain metabolic profiles in aged mice.

KEYWORDS: blueberry, anthocyanins, metabonomics, aged mice, NMR

INTRODUCTION

Normal aging is accompanied by declines in motor and cognitive performance. These declines are amplified in age-related neurodegenerative diseases such as Alzheimer's disease (AD), Parkinson's disease (PD), and amyotrophic lateral sclerosis (ALS). (As the elderly population increases, so will the prevalence of these age-related disorders.[1,2]) To improve the quality of life for the elderly and to alleviate the social and economic burdens imposed by the prolongation of life expectancy, it is crucial to devise strategies to impede or reverse age-related neuronal declines.[1] Dietary supplementation with antioxidant fruits and vegetables can reduce oxidative stress in brain regions and can ameliorate age-related deficits in neuronal and behavioral functions.[3]

Blueberry (BB) is one of the richest sources of anthocyanins, as well as many essential nutrients,[4] that exhibit a wide range of biological effects. It also has one of the highest recorded in vitro antioxidant capacities of various fruits and vegetables tested.[5] Blueberries are relatively low in antioxidant vitamins and minerals; the antioxidant capacity in vitro has been attributed to their high concentration of phenolic compounds, particularly anthocyanins.[6] It was reported that supplementation with a blueberry diet (2% w/w) for 12 weeks improved the performance of aged rats in spatial working memory tasks.[7] Recent research has shown that a blueberry-enriched diet provided cellular protection against oxidative stress and reduced a kainate-induced learning impairment in rats.[8] In our previous study, the aged mice supplemented with blueberry extracts or cyanidin-3-*O*-galactoside from blueberry displayed superior learning performance compared to a control group.[9] However, the mechanisms of anthocyanins from blueberry for improving cognition impairment in the aged mice are still ambiguous.

Nuclear magnetic resonance (NMR) spectroscopy-based metabolomic strategies have been developed for generating comprehensive biochemical profiles of low molecular weight metabolites in biofluids that change in response to internal and external stimuli. In recent years, the number of reports on its application in the field of nutrition has increased, such as the value of metabolites in different biofluids in nutritional metabonomics, the issues of non-nutrient chemicals and large-bowel metabolites, and the linkage of metabonomics with the wider elements of nutrigenomics.[10] On the other hand, this technology has been successfully applied to the neuroscience field. Many efforts have been made to investigate metabolite variation in the brain with aging using ^{1}H NMR.[11] In elderly persons, total *N*-acetylaspartate in semiovale white matter was found to be decreased; however, total myoinositol levels in semiovale white matter and total creatine in frontal lobe typically increased.[12] These results identified that the metabolic changes with aging might be related to age-related cognitive decline during aging. The aim of this study was to investigate and compare the changes of metabonomics of plasma and brain tissue after dietary supplementation with blueberry extracts (BBE) and cyanidin-

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3-*O*-galactoside from blueberry (BBM) in aged mice by NMR-based metabonomics techniques.

MATERIALS AND METHODS

Materials. Blueberry extracts were purchased from Daxinganling Lingonberry Organic Foodstuffs Co. Ltd.; the content of anthocyanidins was 25%. Blueberry monomer from blueberry was cyanidin-3-*O*-galactoside (89.8%), the preparation and structure identification of which were the same as in a previous paper.[9] All other drugs and chemicals, unless specified, were of analytical reagent grade.

Animals and Experiment Protocols. Fifteen male Kunming mice aged 15 months were randomly divided into control group, blueberry extracts (BBE) group, and cyanidin-3-*O*-galactoside (BBM) group. First, the animals were acclimatized for 7 days in individual plastic animal cages in a well-ventilated room at a temperature of 25 ± 3 °C and a relative humidity of 50 ± 10%, with a 12 h light/12 h dark cycle. Food and drinking water were provided ad libitum. The mice in the BBE, BBM, and control groups received intragastric administration of BBE (200 mg/kg/day), BBM (50 mg/kg/day), and an equal volume of saline water (0.9%) for 6 weeks, respectively. At the end of experiment, the mice were decapitated. Plasma samples were collected in heparinized tubes and stored at −80 °C. After decapitation, the brain tissues were rapidly dissected out on ice. The brain tissues for NMR spectroscopic analyses were immediately frozen in liquid nitrogen and stored at −80 °C.

^{1}H NMR Spectroscopy of Plasma Samples. For plasma samples, aliquots of 300 μL of plasma were mixed with 200 μL of D_2O and 100 μL of 0.1% solution of 3-(trimethylsilyl) propionic acid-d_4 sodium salt (TSP) in D_2O and centrifuged at 13000 rpm for 10 min. The supernatants were transferred into 5 mm NMR tubes.

Analysis of plasma samples was carried out on a Varian INOVA 600 NMR spectrometer operating at 599.73 MHz, using a 5 mm triple-resonance probe with *z*-axis gradient at 27 °C.

One-dimensional (1D) spin−echo spectra were recorded using a T2 relaxation edited Carr−Purcell−Meiboom−Gill (CPMG) sequence [90−(−180−)$_n$-acquisition] to attenuate broad signals from proteins and lipoproteins, which resulted in the spectra only with the signals from small metabolites due to their longer transverse relaxation time. A total spin−spin relaxation delay (2*n*) of 320 ms and 128 scans was used. Totals of 64 transients and 64K data points were collected with a spectral width of 8000 Hz. Prior to Fourier transformation, the free induction decays (FIDs) were zero-filled by a factor of 2 and multiplied by an exponential line-broadening function of 1 Hz. Water suppression was applied during the recycle delay.

^{1}H NMR Spectroscopy of Aqueous Soluble Brain Tissue Extracts. Aqueous metabolites from brain tissues were extracted with CH_3CN/H_2O (1:1, v/v) and lyophilized in Labconco Freezone 6. The residue was dissolved by 600 μL of D_2O and 60 μL of 0.1% solution of TSP in D_2O and then centrifuged at 13000 rpm for 10 min. The supernatants were transferred into 5 mm NMR tubes.

Analysis of brain extract samples was carried out on a Varian INOVA 600 NMR spectrometer operating at 599.73 MHz, using a 5 mm triple-resonance probe with *z*-axis gradient at 27 °C. 1D PRESAT spectra were acquired for the brain extract samples. Totals of 64 transients and 32K data points were collected with a spectral width of 9000 Hz. Prior to Fourier transformation, the FIDs were zero-filled by a factor of 2 and multiplied by an exponential line-broadening function of 0.5 Hz. Resulting spectra were manually phased, and the baseline was corrected and referenced to TSP (or TMS) at δ 0.0.

NMR Spectral Data Reduction. The NMR data were phased and baseline corrected, and then the data were reduced to integral regions of equal width of 0.01 ppm using the VNMR program (Varian, Inc.). For plasma samples, the CPMG spectrum was reduced to 400 integrated segments of equal width (0.01 ppm), corresponding to the region δ 4.40−0.40. For brain samples, the spectral region between δ 9.5 and 0.50 was segmented into 900 integrated regions. The integrated data were normalized to the total integrals of each spectrum and were then imported into SIMCA-P 10.0 (UmetricsAB, Umea, Sweden) and preprocessed using centered scaling.

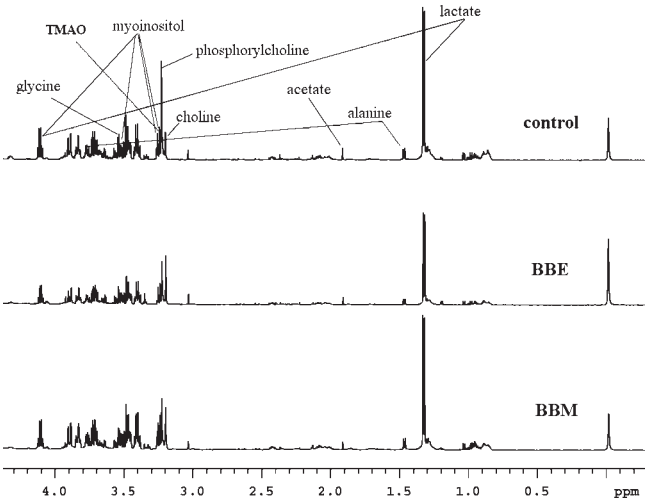

Figure 1. Typical ^{1}H NMR spectra of plasma in mice from control, BBE, and BBM groups. TMAO, trimethylamine oxide.

Multivariate Date Analysis. Data were statistically analyzed by principal component analysis (PCA), partial least-squares discriminant analysis (PLS-DA), or orthogonal signal correction−partial least-squares (OSC-PLS) within the SIMCA package. PCA is a data visualization method that is useful for overviewing relationships or groupings within multivariate data. PCA is performed to examine the dominant intrinsic variation in the data set. Observations are assigned scores according to the variation measured by the principal components with those having similar scores clustering together. When group separation was not satisfied on the basis of PCA, the data were further preprocessed using PLS-DA or OSC to remove the variations not correlated to the group membership. PLS-DA is a supervised extension of PCA used to distinguish two or more classes by searching for variables (*X* matrix) that are correlated to class membership (*Y* matrix).[13] The OSC technique moves irrelevant systematic information from the data set, leading to more powerful PLS models, because OSC uses the matrix *Y* to construct a filter of matrix *X*.[14] The application of this filter was by a corresponding tool provided by SIMCA-P 10.0 software.

Model performance was evaluated using the R^2 and Q^2 parameters, both of which vary between 0 and 1. R^2 provides an indication of how much of the variation within a data set can be explained by the various components of the model. The cumulative score, R^2_{cum}, records how much variation is represented by the total model. Q^2 indicates how accurately the data, either classed or nonclassed, can be predicted, and this term is more relevant to supervised pattern recognition processes.[13] Each point on the score plots represented an individual sample, to reveal specific grouping and the relationship between samples. Each point on the loading plots represented a single NMR spectral region, showing the importance of each metabolite for the variation described.

Statistics. Descriptive statistics of select metabolite levels was performed on the basis of their peak integrals relative to that in the NMR profile. The difference in levels of metabolites in plasma and brain were tested using one-way analysis of varince (ANOVA), with Bonferroni test. *P* values of <0.05 were considered to be statistically significantly different.

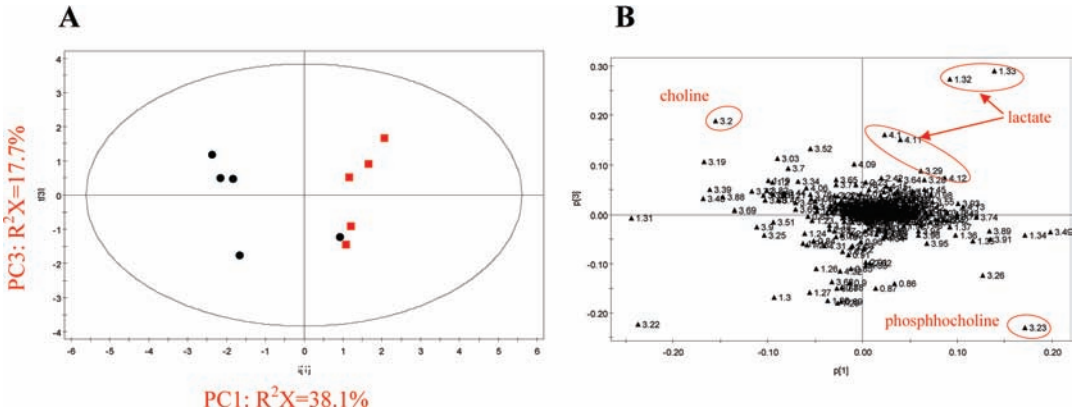

Figure 2. Score plots (A) and loading plots (B) derived from PCA for plasma in BBE (●) and control (■) groups. R^2X_{cum} = 55.8%; Q^2_{cum} = 43.6%.

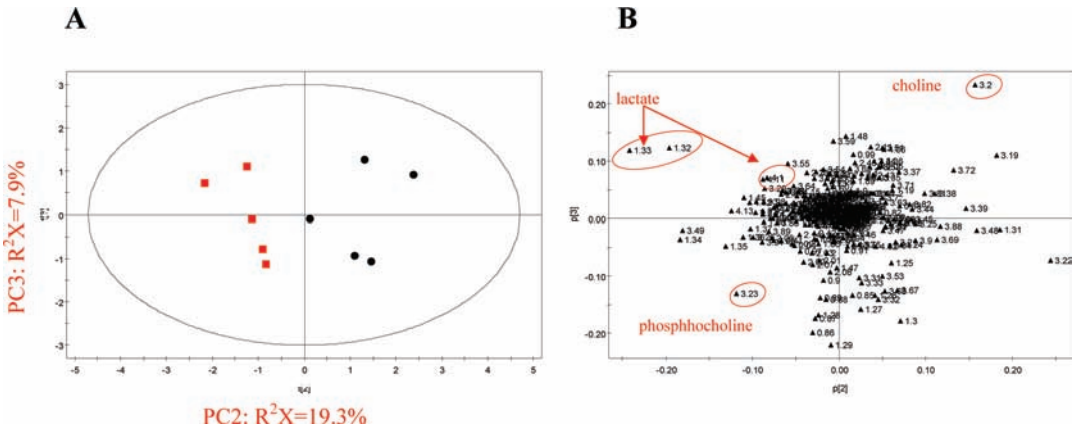

Figure 3. Score plots (A) and loading plots (B) derived from PCA for plasma in BBM (●) and control (■) groups. R^2X_{cum} = 27.2%; Q^2_{cum} = 21.8%.

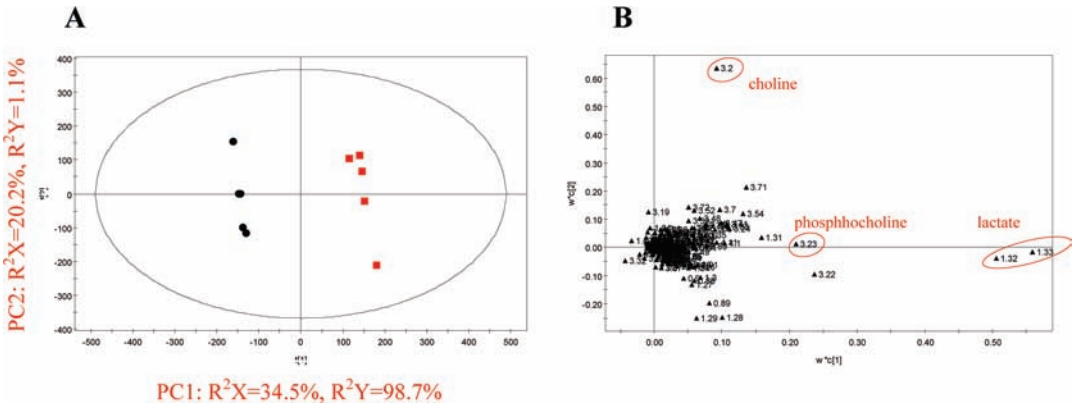

Figure 4. Score plots (A) and loading plots (B) derived from OSC-PLS for plasma in BBE (■) and BBM (●) groups. R^2X_{cum} = 54.7%; R^2Y_{cum} = 99.7%; Q^2_{cum} = 94.4%.

RESULTS

Analysis of Plasma ^{1}H NMR Profiles. Typical ^{1}H NMR spectra for plasma samples from control and BBE- and BBM-treated mice are shown in Figure 1. Visual analysis of the spectra showed that there were distinct variations in three groups. Metabolites that appeared in the ^{1}H NMR spectra of plasma included choline, myoinositol, phosphocholine, acetate, alanine, lactate, trimethylamine oxide (TMAO), and glycine. To detect more subtle supplement-related metabolic differences, pattern recognition techniques were applied. All of the NMR metabolite profiles from different groups were readily separated by PCA or OSC-PLS, indicating that the metabolic characteristics of the three groups were different (Figures 2−4). The PCA score plots (Figures 2A and 3A) showed a clustering of plasma samples related to BBE, BBM, and control groups in the first and second PCs (Q^2_{cum} = 43.6%) or the second and third PCs (Q^2_{cum} = 21.8%). However, the PCA score plot (Figure 2A) showed that one sample in the BBE group was in the control group, compared with the other samples. This sample was excluded from analysis.

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Table 1. Changes and Assignment of the Metabolites in Plasma of Mice among Control, BBE, and BBM Groups[a]

chemical shift[b] (δ)	metabolite	BBE to control	BBM to control	BBM to BBE
1.31−1.33 (d), 4.10−4.12 (d)	lactate	↓	↓	↓
1.4 8(d), 3.77 (q)	alanine	↓	↓	↑/−
1.91 (s)	acetate	↑	−	↓
3.2 (s)	choline	↑	↑	↓
3.23 (s)	phosphocholine	↓	↓	↓
3.26 (s)	TMAO	↓	↓	↓
3.55 (s)	glycine	↓	↓	↓
3.27 (t), 3.53 (t), 3.61 (t), 4.05 (t)	myoinositol	↑	↑	↓

[a] ↑, increase; ↓, decrease; −, no change. [b] s, single; d, doublet; dd, double doublet; m, multiplet; q, quartet; t, triplet.

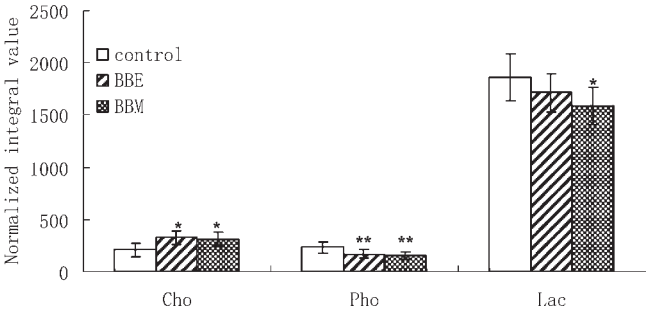

Figure 5. Changes of metabolites in plasma. The results were expressed as mean ± SD. *, $P < 0.05$, **, $P < 0.01$, compared with the control group ($n = 5$). Cho, choline; Pho, phosphocholine; Lac, lactate.

PCA and PLS-DA of plasma profiles from the BBE and BBM groups did not display intrinsic clustering related to BBE and BBM intake in the first three PCs. OCS-PLS was used to maximize the separation between the ^{1}H NMR plasma profiles after BBE and BBM ingestion. OSC-PLS of the plasma endogenous metabolic profiles (Figure 4) showed a clustering of plasma samples related to the BBE and BBM groups in the first and second PCs ($Q^2_{cum} = 94.4\%$). The metabolites contributing to the differences in score plots of Figures 2A, 3A, and 4A were then revealed by analysis of the corresponding loading plots of Figure 2B, 3B, and 4B. The assignments of important endogenous metabolites in plasma samples are presented in Table 1.

In plasma, the metabolites that predominantly contributed to the separation of the control, BBE, and BBM groups were choline, phosphocholine, and lactate (Figures 2B, 3B, and 4B and Figure 5). Acetate, choline ($P < 0.05$), and myoinositol in the BBE group were higher than those in the control group, whereas the levels of alanine, lactate, phosphocholine ($P < 0.01$), TMAO, and glycine were decreased. The levels of choline ($P < 0.05$) and myoinositol in the BBM group were elevated, and acetate showed no changes, compared with that in control. In addition, lactate ($P < 0.05$), alanine, phosphocholine ($P < 0.01$), TMAO, and glycine in the BBM group decrease, compared with the control (Figure 5; Table 1).

Compared with the BBE group, the mice had lower levels of choline, lactate, acetate, myoinositol, phosphocholine, TMAO, and glycine in the BBM group, whereas the level of alanine was a little elevated (Table 1).

Analysis of Brain ^{1}H NMR Profiles. Figure 6 displays three representative ^{1}H NMR spectra of brain samples from control and BBE- and BBM-treated mice. From visual inspection of ^{1}H

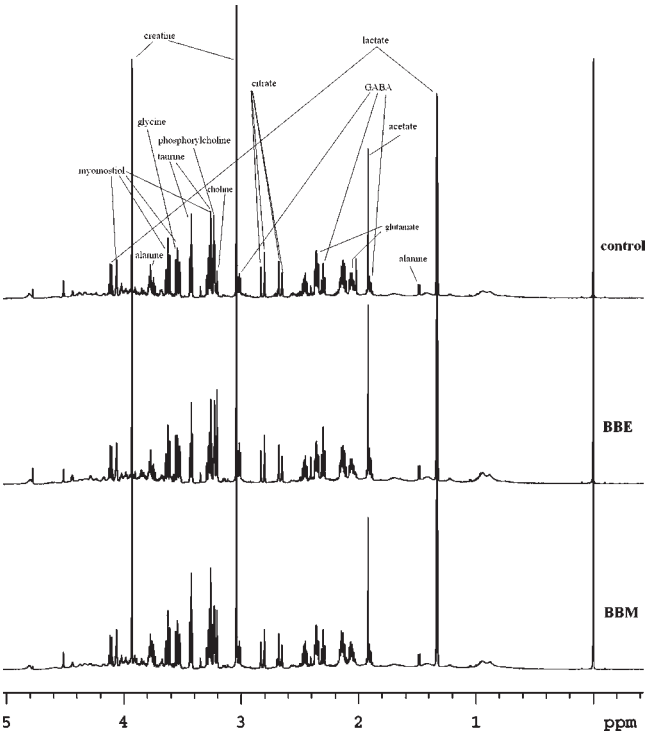

Figure 6. Typical ^{1}H NMR spectra of brain from control nd BBE- and BBM-treated mice. GABA, γ-aminobutyric acid.

NMR spectra of brain samples, the levels of choline and GABA in BBE- and BBM-treated mice were obviously elevated, compared with those in control group. In addition, the signals of -myoinositol, creatine, glycine, taurine, phosphocholine, citrate, glutamate, acetate, alanine, and lactate appeared in the ^{1}H NMR spectra of brains from control and BBE- and BBM-treated mice. To be able to detect more subtle supplement-related metabolic differences, PCA and PLS-DA were performed on all brain metabolite profiles. The PCA score plot (Figure 7A) showed a clustering of brain samples related to BBE and control groups ($Q^2_{cum} = 35.7\%$) in the first and second PCs. The PCA score plot (Figure 9A) also showed a clustering of brain samples related to BBE and BBM groups ($Q^2_{cum} = 33.1\%$) in the first and second PCs. PCA of brain profiles from BBM and control groups did not display intrinsic clustering related to BBM intake in the first three PCs. PLS-DA improved the discrimination between the ^{1}H NMR brain profiles of the BBM and control groups after BBM ingestion. PLS-DA of the brain endogenous

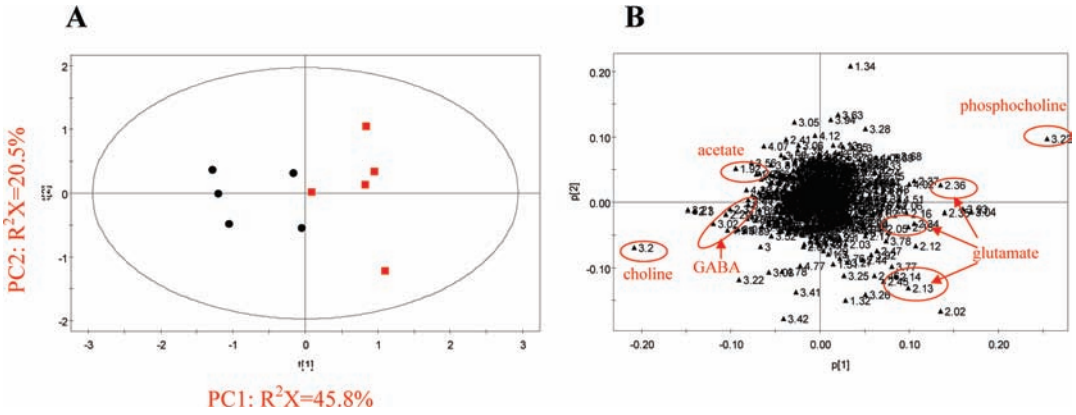

Figure 7. Score plots (A) and loading plots (B) derived from PCA for brain in BBE (●) and control (■) groups. R^2X_{cum} = 66.3%; Q^2_{cum} = 35.7%.

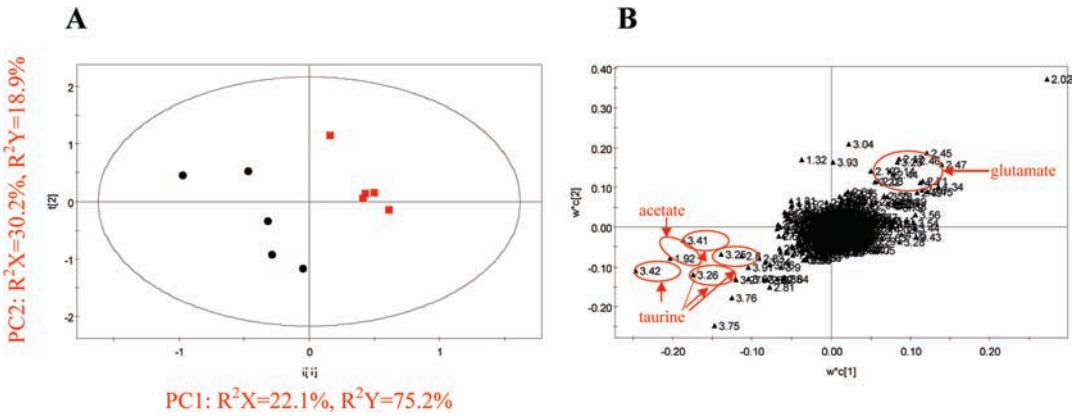

Figure 8. Score plots (A) and loading plots (B) derived from PLS-DA for brain in BBM (●) and control (■) groups. R^2X_{cum} = 52.2%; R^2Y_{cum} = 94.1%; Q^2_{cum} = 48.6%.

Table 2. Change and Assignment of the Metabolites in Brain of Mice among Control, BBE, and BBM Groups[a]

chemical shift[b] (δ)	metabolites	BBE to control	BBM to control	BBM to BBE
1.92 (s)	acetate	↑	↑	–
3.25−3.27 (t), 3.41−3.42 (t)	taurine	↑/–	↑	↑
2.05 (m), 2.13 (m), 2.36 (m), 3.75 (m)	glutamate	↓	↓	↑
3.04 (s), 3.93 (s)	creatine	↓	↓	↑
3.56 (s)	glycine	↑	–	↓
3.23 (s)	phosphocholine	↓	↓	↑
3.20 (s)	choline	↑	↑/–	↓
1.90 (m), 2.29 (m), 3.01 (m)	GABA	↑	↑/–	↓
3.27 (t), 3.53 (t), 3.61 (t), 4.05 (t)	myoinositol	↓	↓	–
2.65, 2.68 (d), 2.80, 2.83 (d)	citrate	↑/–	↑/–	↑/–
1.48 (d), 3.78 (q)	alanine	↑	–	↓
1.31−1.33 (d), 4.10−4.12 (q)	lactate	↓/–	–	↓

[a] ↑, increase; ↓, decrease; –, no change. [b] s, single; d, doublet; dd, double doublet; m, multiplet; q, quartet; t, triplet.

metabolic profiles (Figure 8A) showed a clustering of brain samples related to BBM and control groups in the first and second PCs (Q^2_{cum} = 48.6%). The metabolites contributing to the differences in score plots among control, BBE, and BBM groups were then revealed by analysis of the corresponding loading plots (Figures 7B, 8B, and 9**B**). The assignments of important endogenous metabolites in brain samples are presented in Table 2.

In the brain, the metabolites that predominantly contributed to the separation of the control group and BBE group were choline, phosphocholine, glutamate, and GABA, and the metabolite that predominantly contributed to the separation of the control group and BBM group was taurine (Figures 7B, 8B, and 9B and Figure 10). The levels of choline ($P < 0.001$), GABA ($P < 0.001$), acetate, glycine, citrate, and alanine were elevated in the BBE group relative to the control group, whereas the level of

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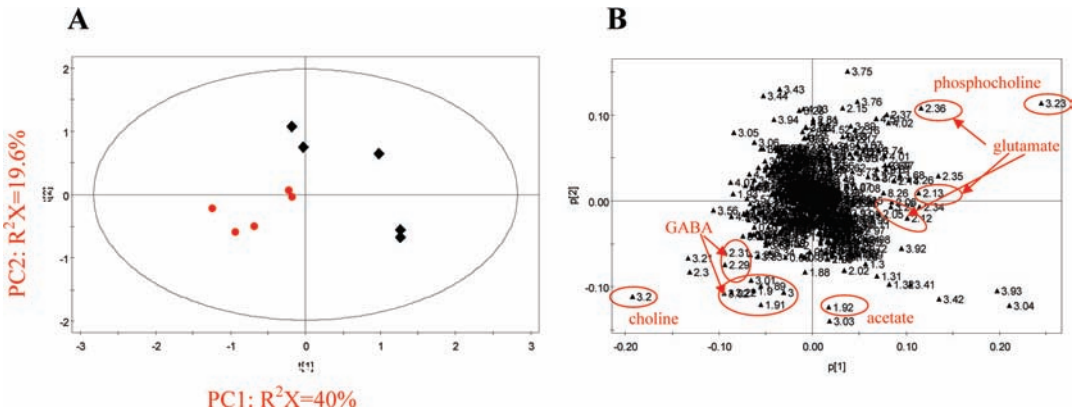

Figure 9. Score plots (A) and loading plots (B) derived from PCA for brain in BBE (●) and BBM (◆) groups. R^2X_{cum} = 59.5%; Q^2_{cum} = 33.1%.

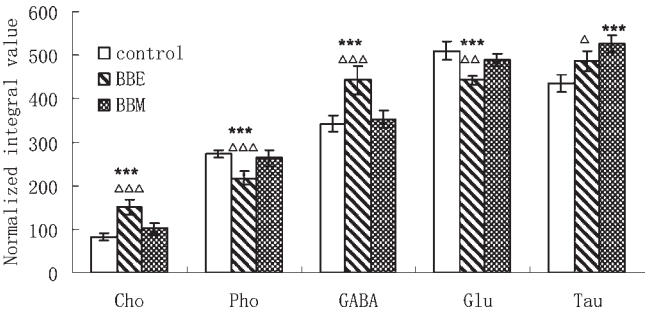

Figure 10. Changes of the metabolites in brain. The results were expressed as mean ± SD. ***, $P < 0.001$, compared with the control group; △, $P < 0.05$, △△, $P < 0.01$, and △△△, $P < 0.001$, compared with the BBM group ($n = 5$). Glu, glutamate; Tau, taurine.

glutamate, creatine, phosphocholine, myoinositol, and lactate were degraded. Taurine in the BBE group was a litter higher compared with the control group. The level of taurine in the BBM group was obviously increased ($P < 0.001$) compared with the control group. Choline, acetate, GABA, and citrate in the BBM group were higher than in the control group. In addition, compared with the control group, glutamate, creatine, phosphocholine, and myoinositol in the BBM group were decreased. Furthermore, no differences were found in those highly contained metabolites between the BBM and control groups, such as glycine, alanine, and lactate (Figure 10; Table 2).

Moreover, compared with the BBE group, the BBM group had higher levels of glutamate ($P < 0.01$), taurine ($P < 0.05$), creatine, phosphocholine ($P < 0.001$), and citrate but lower levels of choline ($P < 0.001$), GABA ($P < 0.001$), glycine, alanine, and lactate. For acetate and myoinositol, no differences between the BBM and BBE groups were found (Figure10; Table 2).

DISCUSSION

In this study, using a combined approach of ^{1}H NMR spectroscopy and multivariate analysis, we found that dietary supplementation with BBE and BBM led to profound metabolic changes of plasma and brain tissue in aged mice. The effects of BBE on plasma and brain metabolism were similar to the experimental results of BBM (Figures 5 and 10; Tables 1 and 2). In plasma, the metabolites that were markedly changed in the BBE and BBM groups were lactate, choline, and phosphocholine, compared with the control group (Figure 5). In the brain, the metabolites that were significantly changed in the BBE group were choline, phosphocholine, glutamate, and GABA, compared with the control group, and the metabolite of the brain that was obviously changed between the control group and the BBM group was taurine. Moreover, compared with the BBM group, the metabolites of the brain that were distinctively changed in BBE group were choline, phosphocholine, taurine, glutamate, and GABA (Figure 10).

It is known that energy supply requires almost entirely the oxidative metabolism of glucose in mitochondria, and when energy demands transiently exceeded the rate of oxidative metabolism, lactate was produced to supply energy as a result of glycolytic processes.[15] An increased lactate level is related to the reduced use of pyruvate in the citric acid cycle and the increase of anaerobic glycolysis. During aging, oxidative stress may increase, which in turn increases the activities of lactate dehydrogenase[16] and thus induces the increment of the lactate level. It was reported that anthocyanins show strong antioxidative activity.[4−6] Therefore, the levels of lactate in plasma of BBE- and BBM-treated mice appear to be degraded through the reduction of oxidative stress, the enhancement of mitochondria function, or the inhibition of the activities of lactate dehydrogenase after supplementation with BBE or BBM.

Choline has been proved to possess positive effects in different neurological disorders that are associated with cognitive deficits. Choline is synthesized from serine and methionine and serves as a precursor for acetylcholine (ACh) and phosphatidylcholine. ACh, a well-known cholinergic neurotransmitter, plays crucial roles in transmitting important biological information in both the central and autonomic nervous system and neuromuscular junctions.[17] ACh is synthesized by choline acetyltransferase in the cholinergic neuronal cells. The ACh released in synaptic clefts is rapidly decomposed by acetylcholine esterase to choline and acetic acid.[18] Choline acetyltransferase activity and ACh release are known to decrease with aging.[19] In this study, we observed that choline and acetate levels in the brains of BBE- and BBM-treated mice were increased (Table 2), compared with those in control group. The results suggested that BBE and BBM may promote the metabolic cycle of acetylcholine. Another result in this study was that choline was increased in plasma from the BBE and BBM groups, compared with the control group. Plasma choline constitutes an important source of choline for neuronal acetylcholine synthesis and for producing phospholipids, an essential component of all membranes. Elevations in circulating choline levels also stimulate acetylcholine synthesis and release and enhance cholinergic neurotransmission.[20]

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Phosphocholine is a precursor for phosphorylcholine (PCHO), which is an essential component of neuronal membranes. Various studies have reported that PCHO content was induced by some different regulatory mechanisms, including the conversion of PCHO into CDP-choline by cytidyltransferase, conversion of choline to phosphocholine by choline kinase, PCHO hydrolysis to choline by an excess of alkaline phosphatase, and so on.[21,22] In our experiments, phosphocholine was decreased in the brain and plasma of BBE- and BBM-treated aged mice. The mechanism of variation of phosphocholine will need further research.

GABA is an inhibitory neurotransmitter that is widely distributed in the neurons of the cortex. GABA contributes to motor control, vision, and many other cortical functions.[23] Glutamate is a major excitatory neurotransmitter that is involved in a number of cerebral functions which become altered with age, such as learning and memory,[23,24] emotion and motivation,[25] and motor functions.[26] An increased susceptibility to glutamate-induced toxicity with age has also been described,[27] which may be due in part to a reduction in the concentration of antioxidants.[28] GABA is intimately related to glutamate/glutamine cycling. In the cycling, glutamate is converted to glutamine and released from astrocytes. Thereafter, the glutamine is converted to GABA in GABAergic neurons.[29] On the other hand, glutamate plays an important role in maintaining the GABA level in the brain by conversion to GABA by the GABA-biosynthesizing enzyme and glutamic acid decarboxylase (GAD).[30] In the results shown in Table 2 and Figure 10 of the present study , GABA was increased, whereas glutamate was decreased in the brains of BBE- and BBM-treated mice, which might result from the activity of increased GAD after supplementation with BBE and BBM. In addition, because GABA levels in brain tissue mainly reflected the amount of GABAergic neurons, the increased GABA indicated an enhancement of GABAergic neurons in aged mice treated with BBE and BBM, which would have a significant impact on the function of the brain and memory processing.

Taurine, a sulfonated β-amino acid, presents in high concentrations in the mammalian central nervous system (CNS).[31] It has been suggested to be neuroprotective. Its effects include calcium modulation, apoptosis inhibition, and antioxidant properties. Growing evidence shows that taurine may function as a potent candidate of inhibitory neurotransmitter or modulator to regulate neuronal activity in many cerebral areas.[32] As an inhibitory amino acid in the CNS, taurine could activate glycine receptors or GABA receptors. Thus, maintenance of taurine levels in aged brains could be implicated in the improvement of behavioral learning observed in aged mice. Compared with young rats, the taurine concentrations in old rats are significantly decreased in the brain.[33] In the present experiments, taurine was at higher levels in the brains of BBM-treated mice. The results suggested that BBM probably improved cognition impairment by enhancing the level of taurine in the brains of the aged mice.

In conclusion, this study has demonstrated that BBM supplementation was similar to BBE supplementation in the effects of metabolism in aged mice. The changed metabolites were mainly lactate, choline, phosphocholine, taurine, glutamate, and GABA. Metabolism analysis indicated that supplementation with blueberry extracts or cyanidin-3-*O*-galactoside from blueberry induced changes in the endogenous plasma and brain metabolic profiles in aged mice, confirming the bioactivity of this fruit component. The results will aid in the understanding of the mechanism of blueberry supplementation, which improves cognitive impairment and neurodegenerative diseases.

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