



## Case Report

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# Immediate Decrease in $\gamma$ -AminoButyric Acid after Caffeine Intake in Adolescents: a Preliminary MRS Study

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In adolescents, sleep deprivation problem is getting worse, and increased caffeine consumption is considered to relieve the stress caused by sleep deprivation and academic burden. In this study, immediate neurologic effects of caffeine intake on adolescents were evaluated in three high school students using the  $\gamma$ -aminobutyric acid (GABA)/creatinine ratio on magnetic resonance spectroscopy (MRS). MEGA-PRESS MRS and TE 135 ms single voxel MRS were performed in the anterior cingulate cortex before and after drinking a cup of coffee, which contained 104 mg of caffeine. GABA and creatinine were measured on LCModel 6.3, respectively. In all three students, GABA/creatinine ratios were decreased after caffeine intake. The GABA/creatinine ratios obtained before caffeine intake were decreased after caffeine intake in all the three adolescents. In this preliminary study, caffeine intake caused an immediate decrease in the GABA/creatinine ratio in the brain and it may be related to the neurologic effects of caffeine on an adolescent's brain.

**Keywords:** Gamma aminobutyric acid; Brain; Caffeine; Magnetic resonance spectroscopy

## INTRODUCTION

Sleep deprivation is a condition characterized by lack of sleep. In adolescents, sleep deprivation problem is getting worse because of various causes such as academic stress, social dysfunction or mood changes. At the same time, consumption of caffeine has increased in this age group to endure sleep deprivation. Caffeine alleviates fatigue and helps the adolescents stay awake for a longer period by temporally increasing glucose metabolism in the brain. However, because it is a stimulant of the central nervous system as well as an adenosine receptor antagonist, excessive consumption of caffeine can result in palpitation, epigastric tenderness, headache, anxiety and insomnia. Ministry of Food and Drug Safety recommends maximum caffeine consumption of 2.5 mg/kg for teenagers (1).

It is believed that chronic effects of caffeine may be related to long-term decrease in  $\gamma$ -aminobutyric acid (GABA) (2). GABA is the primary inhibitory neurotransmitter which alleviates anxiety and induces sleep. Therefore, GABA decline causes anxiety and it may even result in a psychotic disorder such as schizophrenia or major depressive disorder. In particular, patients with major depressive disorder show a significantly decreased

*in vivo* GABA rate (3). To the best of our knowledge, no research has been conducted on the immediate response of the human brain, especially in adolescents, after caffeine intake.

In this report, we demonstrated the immediate neurological effects of caffeine in three high school students using the GABA/creatine ratio on magnetic resonance spectroscopy (MRS). We also evaluated the changes in vital signs before and after the intake of caffeine. GABA signals were obtained at the anterior cingulate cortex (ACC) of each student on MRS. Being a regulator of cognitive-emotional processing, the ACC in the limbic system serves as a critical part of attention focusing (4). GABA signals were measured using a technique known as MEGA-PRESS (MEshcher-GARwood Point RESolved Spectroscopy), which has gained popularity as a GABA measurement tool in recent years. MEGA-PRESS separates GABA signals from signals of other metabolites by giving priority to GABA's known couplings (5). To prevent inaccurate measurement of GABA because of interindividual variance, the GABA/creatine ratio was used instead of the true value of GABA (6).

### CASE REPORT

Two male and one female healthy adolescents (age 16-17 years, mean age 16.3 years) underwent brain MRS on a 3T MR scanner with 32 channel head coil (MAGNETOM Skyra, Siemens Healthcare, Germany). They did not have any history of allergy to caffeine and they had enough sleep before the examination. MEGA-PRESS MRS and echo time (TE) 135 ms single voxel MRS were performed before and after drinking a cup of coffee, which contained 104 mg of caffeine (Fig. 1). MRS was performed before and 90 minutes after caffeine intake.

GABA measurement was performed using MEGA-PRESS

pulse sequence from a voxel volume of 26.25 cc (voxel dimension 3.5 x 3.0 x 2.5 cm) in the ACC with use of the following acquisition parameters: repetition time (TR), 2000 ms; TE, 68 ms; spectral width, 2000 Hz; number of oversampled data, 2048; number of signal averages, 256. GABA resonance at 3.01 ppm was detected by application of a refocusing pulse at 1.9 ppm during ON spectra and at 7.5 ppm during OFF spectra. Water signal suppression was achieved with the chemical shift selective imaging technique. First- and second-order shimming was performed using a pencil beam-volume, resulting in water line widths of < 16 Hz for FC, < 12 Hz for PC, and < 13 Hz for OC. Scan time was approximately 9 minutes.

Single voxel H-MRS with TE 135 ms was obtained at the same voxels in the ACC with use of the following parameters: TR, 2000 ms; number of samples, 64. In addition to MRS, T1-weighted structural images were obtained by a magnetization prepared rapid acquisition gradient echo (MPRAGE) sequence with use of the following parameters: TR = 2000 ms, TE = 3.55 ms, flip angle = 8°, slice thickness = 1 mm and acquisition matrix = 256 x 256. In addition to MRS, T1-weighted structural images were obtained by a magnetization prepared rapid acquisition gradient echo (MPRAGE) sequence with use of the following parameters to position the voxels on MRS: TR = 2000 ms, TE = 3.55 ms, flip angle = 8°, slice thickness = 1 mm and acquisition matrix = 256 x 256.

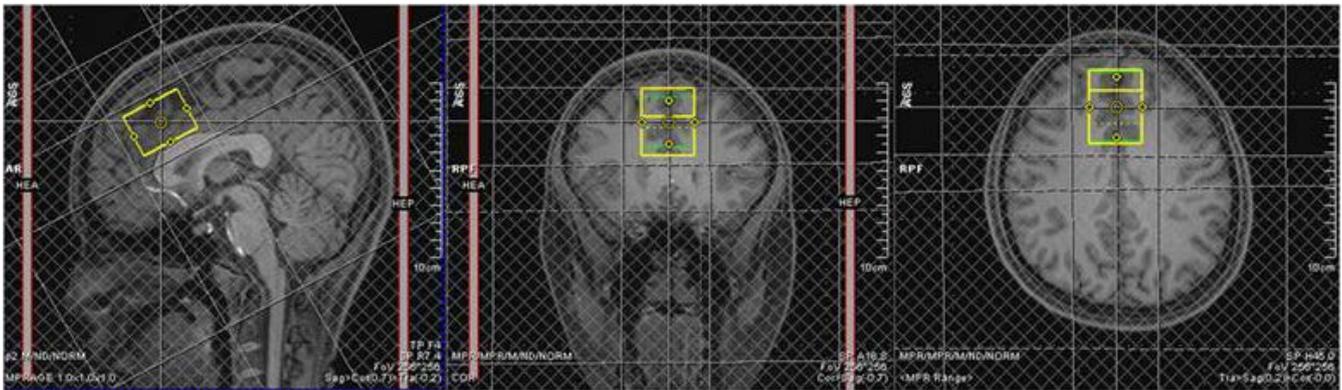
The change in GABA was assessed by the GABA/creatine ratio before and after caffeine intake. GABA signal was displayed as a double peak at 3 ppm. GABA and creatine were measured on LC Model 6.3, respectively (7). In addition, various vital signs of the three adolescents were measured before and after caffeine intake; blood pressure, heart rate, body temperature, and oxygen saturation in blood. These parameters were measured just before MRS scanning.

**Table 1. Changes in Vital Signs in Three Subjects Before and After Caffeine Intake**

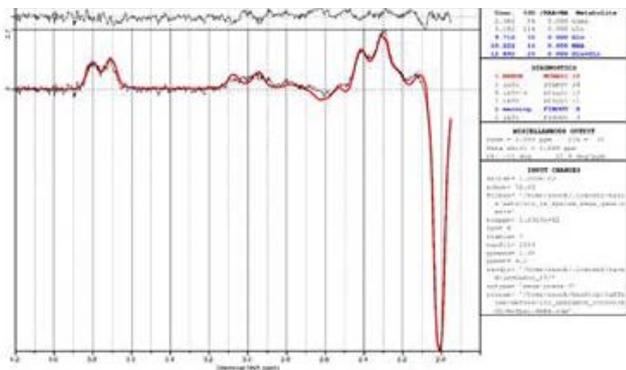
Subjects	Before caffeine intake					After caffeine intake				
	Maximal blood pressure (mmHg)	Minimal blood pressure (mmHg)	Heart rate per minute	Body temperature (°C)	Oxygen saturation (%)	Maximal blood pressure (mmHg)	Minimal blood pressure (mmHg)	Heart rate per minute	Body temperature (°C)	Oxygen saturation (%)
1	102	58	61	36.6	99	113	66	65	36.8	100
2	101	64	78	36.6	98	104	68	75	37.0	100
3	125	60	70	36.1	97	139	62	72	36.5	100
Mean	109.3	60.7	69.7	36.4	98	118.7	65.3	70.7	36.8	100

Table 1 demonstrates the changes in vital signs in these three subjects before and after caffeine intake. Maximal and minimal blood pressures, body temperature, and oxygen saturation rose after caffeine intake in all the three subjects. There was no significant vital sign change or symptom after caffeine intake. Table 2 shows the changes in the GABA/

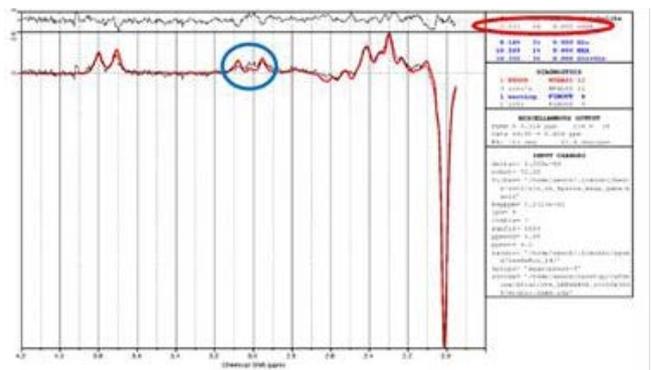
creatine ratio before and after caffeine intake. In all the three subjects, the GABA/creatine ratio was decreased after caffeine intake, respectively.



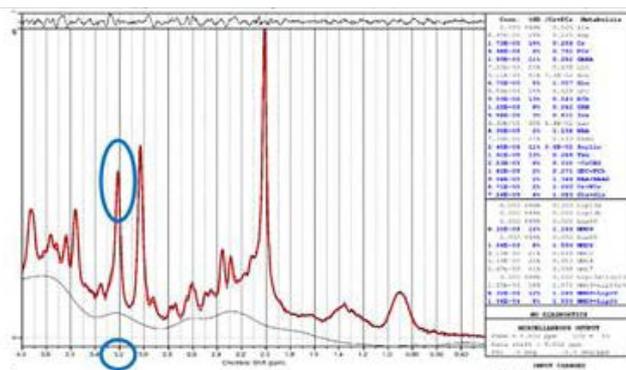
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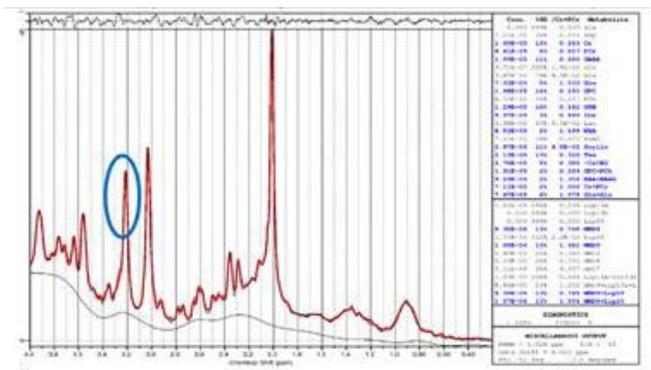
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e

**Fig. 1.** Measurement of the GABA/creatine ratio on brain MRS (Subject 1). (a) Brain MRS for measuring GABA at the anterior cingulate cortex. (b) GABA rate before caffeine intake. (c) GABA rate after caffeine intake. (d) Creatine before caffeine intake. (e) Creatine after caffeine intake.

**Table 2. Changes in the GABA/Creatine Ratio in Three Subjects Before and After Caffeine Intake**

Subjects	Before caffeine intake			After caffeine intake		
	GABA	Creatine	GABA/creatine ratio	GABA	Creatine	GABA/creatine ratio
1	2.382	6.71	0.3549925	2.037	7.11	0.2864979
2	2.774	7.56	0.3669312	2.930	8.46	0.3463357
3	2.415	8.53	0.2831184	1.838	8.37	0.2195938
Mean	2.524	7.60	0.3350141	2.268	7.98	0.2841425
Standard deviation	0.217422	0.91065910	0.0453376	0.5815946	0.7547847	0.0634038

GABA = Gamma aminobutyric acid

## DISCUSSION

This report focuses on the immediate change in GABA level after caffeine intake in adolescents. Using brain MRS, we could measure the GABA/creatine ratio in the ACC before and after caffeine intake. As MRS only shows the relative value of chemical substances, GABA rate and the GABA/creatine ratio were used to assess the immediate effect of caffeine. Our report showed that the relative GABA/creatine ratio had a similar tendency toward being reduced just after caffeine intake in all the three subjects. However, this study could not control confounding factors which can have an effect on the GABA level in brain, such as sex or female menstrual cycle, etc., and further large-scale study is needed to confirm the relationship between caffeine and GABA.

The ACC lies in a unique position in the brain, with connections to both the "emotional" limbic system and the "cognitive" prefrontal cortex; therefore, it plays an important role in the integration of neuronal circuitry of affect regulation (8). Because GABA acts as an inhibitory neurotransmitter, a decrease in the GABA level in the ACC might be related to anxiety or excitement in adolescents. Although the results of this study showed an immediate and instant reaction of the human body, if these reactions continue consistently and repetitiously, there is enough possibility of a negative influence of chronic caffeine intake on adolescents.

This study suggested the possibility of an immediate decrease in the GABA level after caffeine intake. It could become a stepping stone for providing a biochemical explanation for the specific mechanism or causes of the

effect of caffeine on the adolescent brain.

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