Metabolic changes detected by *in vivo* proton magnetic resonance spectroscopy in the striatum of rats treated with alteration of dietary sulfur amino acids content

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Introduction Sulfur amino acids (SAA) play a central role in many diverse functions including digestion, osmotic regulation, detoxification, hormonal regulation, biological methylation, cell growth regulation and redox regulation. SAA deficiency could induce complex metabolism perturbation. It is shown recently that glutathione (GSH) concentration decreased and cysteine (Cys) /cystine (Cyss) redox increased in plasma induced by SAA deficiency[1]. However, the specific effects of deficient dietary SAA content on brain is unknown. In this study, the changes in the level of metabolites in the rat bain through longitudinal experiments under the SAA deficient diet were monitored by means of magnetic resonance imaging (MRI) and magnetic resonance spectroscopy (MRS).

Materials and Methods Five Sprague-Dawley male rats (300±20 g) were housed in individual cages with free access to water and food. After a 7-day acclimation period with standard pelleted rat food, the rats were fed SAA containing food (+SAA, TD.99366, Harland-Teklad) and SAA deficient food (-SAA, TD.04231, Harland-Teklad). In –SAA diet, there is no cystine and the amount of methionine is 20% of that in +SAA diet. The overall dietary schedule consists of 2-week +SAA food (+SAA1), 2-week -SAA food (-SAA1), 2-week -SAA food (-SAA2), 2-week +SAA food (+SAA2) and 2-week +SAA food (+SAA3). Changes of rat weight and daily food intake were monitored every 3 days. MR experiments were carried out on a 9.4 T/30 cm Bruker Biospec scanner with volume coil excitation and surface coil reception. The animal was initially anesthetized with 5% isoflurane in O2:N_2O (3:7), which was reduced to 1.5~2% for maintenance. T_2-weighted images were acquired by a spin echo sequence with TE of 40 ms, TR of 2.5 s and FOV of 3 cm×3 cm. Localized ^{1}H spectroscopy was acquired from the right striatum using a STEAM sequence with TE of 4 ms, TM of 15 ms, TR of 5 s, and with VAPOR water suppression (256 averages) and without water suppression (16 averages). All the images and spectra were obtained every two weeks. MRS data were analyzed by LC Model software with a TE-specific basis set and using the water signal as the internal reference. The ratio of integrated metabolite peak to that of creatine (Meta/Cr) was used for principal components analysis (PCA). For each rat, Meta/Cr obtained at +SAA1 was set as baseline. Statistical analysis was performed by paired student's *t*-test.

Results Figure 1 shows the result obtained from PCA for all the groups fed with +SAA and –SAA diet. The score plot of PCA shows that +SAA group and –SAA group can be separated completely (Figure 1, a). The loading plot shows the glutamine (Gln), glutamate (Glu), glutamate+glutamine (Glu+Gln) and Taurine (Tau) are the main contributors to this discrimination (Figure 1, b). Figure 2 is the longitudinal changes of relative Gln, Glu, Glu+Gln and Tau levels in responding to the alterations of diets. Compared to +SAA1 period, the relative level of Glx in the striatum of rat brain increased significantly in –SAA1 and –SAA2 periods and decreased significantly in the following +SAA2 and +SAA3 periods. Gln is more sensitive than Glu in responding to the alteration of SAA content in diet. The relative level of Tau decreased significantly without SAA intake and recovered with continuous treatment of SAA containing food. The changes of daily body weight and food intake of the rats fed with +SAA diet and –SAA diet were shown in Table 1. Daily food intake with SAA deficient diet was significantly lower than with SAA containing diet. The maximum increase of the daily body weight was in the +SAA1 period, the least increase in the –SAA1 and –SAA2, and the medium in the +SAA2 and +SAA3.

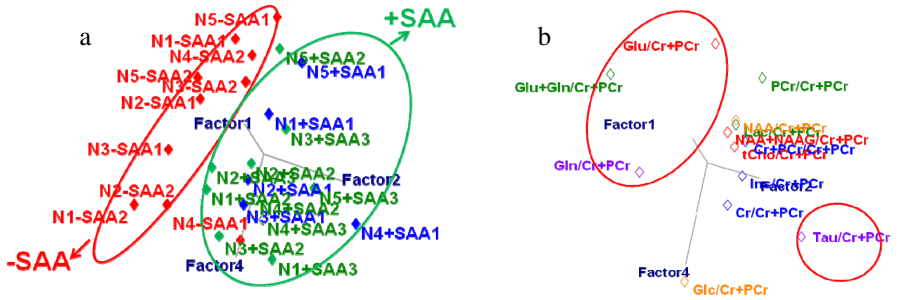

Figure 1 PCA analysis result. The groups fed –SAA diet and +SAA diet can be separated completely shown as the scatter plot (a), the main contributors for this separation is Glx and Tau shown as the loading plot (b).

Discussion The main finding in this study is that the alteration of dietary SAA content can change the metabolite levels in the striatum of rat brain, such as Glx and Tau. Glutamine and taurine are the most sensitive substances affected by this alteration. Taurine is the most abundant intracellular amino acid in the human body. It plays an important role in cell membrane stabilization, modulation of intracellular calcium levels, osmoregulation and detoxification. In healthy humans, dietary foods containing methioine and cysteine are the main sources of taurine[2]. The present data in this study shows that SAA deficient diet could induce an obvious decrease of taurine concentration in the brain, which would consequently lead to many biological and physiological dysfunctions. Although daily food intake in –SAA period was deceased about 15% compared with +SAA period, the daily body weight change was almost decreased by 82% compared with +SAA1 and 70% compared with +SAA2/3. This catabolic response is due to the increase of muscle protein degradation induced by deficiency of the essential amino acid methionine in the diet, associated with decreased overall food intake[1,3]. Cystine is transported in a sodium independent manner across the astroglial cell membrane in exchanges for glutamate via the transport system Xc^-[4]. In SAA deficiency period, cystine concentration in plasma decreased resulting in the increase of glutamate concentration in brain for the exchange mechanism. Consequently, glutamine synthesis in brain could be up-regulated by the increase of glutamate concentration. It is more interesting that the concentration of Glx and Tau went back to their initial values after the follow-up treatment of +SAA diet of 2 weeks. However, whether the biological and physiological functions in the organism affected by the SAA deficiency also recovered needs further study.

References: 1) Nkabyo YS et al, J Nutr. 2006 May;136(5):1242-8; 2) Lourenço R et al, Nutr Hosp. 2002 Nov-Dec;17(6):262-70; 3) Barnes DM et al, J Nutr. 1995 Oct;125(10):2623-30; 4) Bannai S, J Biol Chem. 1986 Feb 15;261(5):2256-63.

Table 1 Changes of body weight and daily intake in rats fed +SAA and –SAA diet (*g/day*)

	+SAA1	-SAA1,2	+SAA2,3
ΔWeight (g/day)	5.06±1.04	0.89±0.31**	3.01±0.69**,##
Food (g/day)	22.9±2.06	19.34±1.98*	20.98±0.98

*: p< 0.05 **: p< 0.01 (Compared with +SAA1 by paired student t-test)
##: p< 0.01 (Compared with –SAA1,2 by paired student t-test)

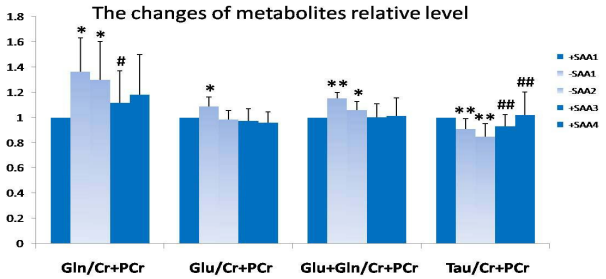

Fig. 2 Changes of metabolites relative levels in responding to the alteration of SAA content in diet. (*p<0.05 compared to +SAA1, #p<0.05 compared to –SAA1 by paired student T-test).