Effect of Creatine Supplementation on AGAT Expression and Metabolic Intermediates in GAMT-Deficient Mice

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Introduction

Cerebral creatine (CT) depletion is diagnostic for the two biosynthetic creatine disorders; AGAT- and GAMT-deficiency. Treatment with oral CT supplementation is used to replenish CT levels. It is also in part successful at reducing the elevated, neurotoxic, guanidinoacetate (GAA) levels unique to GAMT deficiency. GAA reduction likely occurs through CT-mediated AGAT suppression.

Objectives

1. Evaluate the effect of 10-week creatine supplementation (2% and 4%) on AGAT expression and creatine metabolites in organs: kidney, heart, liver, calf muscle, and brain of GAMT-deficient mice
2. Elucidate the mechanism of creatine-mediated AGAT-suppression
3. Evaluate the efficacy as a therapy for metabolite normalization

Methods

Wild type (WT), GAMT heterozygous (GAMT HET) and GAMT-deficient (GAMT MUT) mice, 10-12 weeks old, were fed with 2% or 4% creatine-containing mouse chow for 10 weeks. Mouse urine, blood and organs were collected after 10 weeks. Creatine and GAA in mouse urine, plasma and tissues were analyzed by LC-MSMS. AGAT protein level was evaluated by Western blot. AGAT enzyme assay was performed by LC-MSMS.

Creatine and GAA in Mouse Urine and Plasma

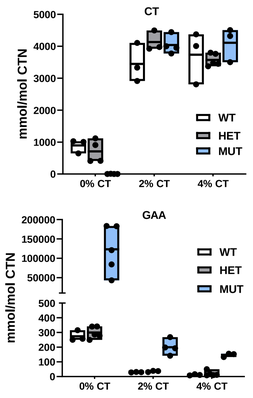

Fig. 1 Mouse urine creatine (CT) and GAA after ten weeks treatment with 2% or 4% CT

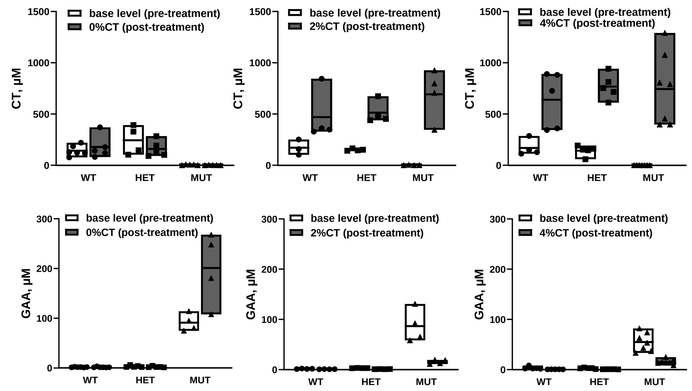

Fig. 2 Mouse plasma creatine (CT) and GAA after ten weeks treatment with 2% or 4% CT

Creatine was increased and GAA decreased in urine and plasma after 2% or 4% creatine treatment

AGAT in Mouse Tissues

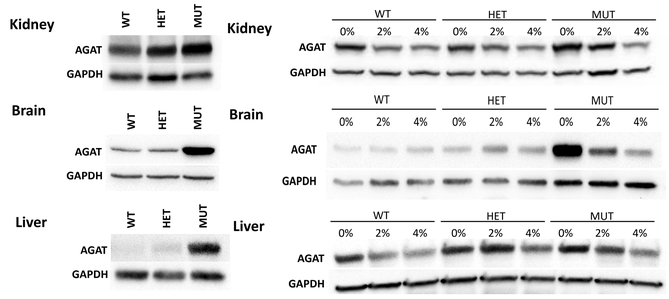

Fig. 3 AGAT protein level in kidney, brain, and liver of WT, GAMT HET, and GAMT MUT mice

Fig. 4 AGAT protein level after ten weeks treatment with 2% or 4% creatine (CT)

AGAT protein expression is higher in GAMT MUT and GAMT HET compared to WT mice. After 2% or 4% creatine treatment, AGAT level is reduced.

Creatine and GAA in Mouse Tissues

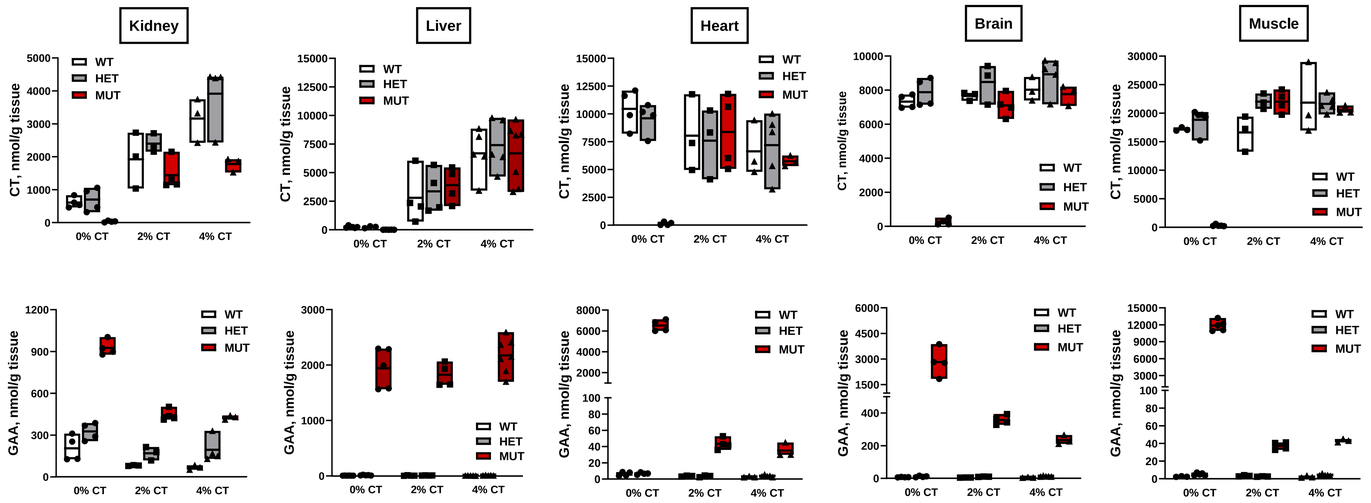

Fig. 5 Creatine (CT) and GAA in WT, GAMT HET and GAMT MUT mice tissues after ten weeks treatment with 2% CT and 4% CT.

After 2% or 4% creatine (CT) treatment, WT mice showed increase of CT in kidneys and liver, and there was no change in brain, heart and muscle. GAMT MUT mice showed increase of CT in all organs. GAA was reduced in all organs, except GAMT MUT liver.

AGAT Activity

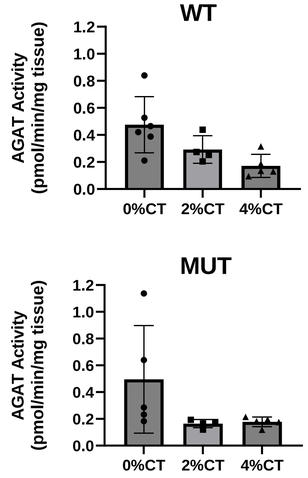

Fig. 6 AGAT enzyme activity in WT and GAMT MUT mice after ten weeks treatment with 2% and 4% creatine (CT).

AGAT enzyme activity was reduced in WT and GAMT MUT mice after the treatment.

Conclusion

We demonstrate the efficacy of creatine on reduction of GAA in urine, plasma and organs (except liver) of WT, GAMT HET, and GAMT MUT mice. Increase of creatine exhibits a noticeable inhibitory effect on AGAT expression and its activity.