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Letter to the Editor

Lowering methylation demand by creatine supplementation paradoxically decreases DNA methylation

Dear sir,

With great interest we have read the recent paper by Heil et al. describing the DNA-methylation status in patients with cystathionine beta-synthase (CBS) deficiency [1]. Unexpectedly, these authors did not find an altered methylation status in patients with CBS, where significant higher total plasma homocysteine (tHcy) and *S*-adenosyl homocysteine (AdoHcy) concentrations were observed in these patients, which is considered as a potent inhibitor of all transmethylation reactions.

In line with these findings and following our previous published work, we would like to present in this letter our findings regarding DNA-methylation in response to creatine supplementation in an animal model of renal failure.

Previously we described a decrease in tHcy concentrations and a folate-sparing effect of creatine in a surgical animal model of renal failure, by lowering methylation demand through inhibition of endogenous creatine synthesis upon dietary creatine supplementation [2]. tHcy concentrations were found to decrease by 20% whereas plasma folic acid and liver tetrahydrofolate concentrations were increased by 42% and 23%, respectively.

In this study, Wistar rats ($n = 36$) were allocated to four study groups according to the surgical procedure (subtotal nephrectomy/sham-operation) and diet (creatine/control) [2]. Using capillary electrophoresis [3] we determined 5-methylcytosine concentrations in liver tissue, indicative for global DNA-methylation status after DNA-extraction, purification and acid hydrolysis.

Global DNA-methylation, expressed as the 5-methylcytosine/cytosine ratio was found to decrease by 22% upon creatine supplementation ($2.69 \pm 0.23\%$ vs. $2.11 \pm 0.13\%$; $p = 0.03$). Table 1 illustrates the general linear model for global DNA-methylation. Creatine treatment was found to be inversely associated with DNA-methylation. No significant effect of body weight, renal failure or food intake or folic acid was observed. No correlation between global DNA-methylation and tHcy-concentrations were found ($r = 0.24$, $p = 0.17$).

Heil et al. [1] were unable to demonstrate lower DNA-methylation in CBS-patients, which they explained though the preserved *S*-adenosyl methionine (AdoMet)/AdoHcy ratio whereas marked higher tHcy and absolute AdoHcy concentrations were noted. Previously, two reports on patients with AdoHcy hydrolyse deficiency observed very high plasma AdoHcy concentrations, but paradoxically global DNA-hypermethylation instead of DNA-hypomethylation [4,5]. These data suggest that another mechanism could be of importance in determining DNA-methylation status.

Table 1
Results of the fixed effects model fit for DNA-methylation

DNA-methylation ~	β(SE)	*t*-value	Significance
Uremia	0.3601 (0.1840)	1.9571	0.0652
Treatment (Creatine vs. Placebo)	−0.4237 (0.1550)	−2.7339	0.0132
Body weight	0.0007 (0.0059)	0.1132	0.9110
Food intake	−0.0203(0.0438)	−0.4628	0.6488
Plasma folate	0.0282 (0.0157)	1.7966	0.0883
Plasma vitB12	0.0004 (0.0009)	0.4673	0.6456

In vitro experiments have demonstrated a decrease in DNA-methyltransferases during differentiation of myoblast cell lines, together with a rise in creatine kinase activity [6]. In regard to creatine supplementation, DNA-methylation could be influenced through the effect on DNA-methyltransferases by altering the cell energetic metabolism. On the other hand, folic acid could have a direct effect on DNA-methyltransferase activity or the DNA-demethylation, independent of the AdoHcy concentrations or methylation demand.

We conclude that in line with the published reports [1,4,5], our data suggest that the determinants of global DNA-methylation are not fully understood. Especially, the importance of the methylation demand and the AdoMet/AdoHcy ratio should reconsidered in determining DNA-methylation and the subsequent role in carcinogenesis and atherosclerosis.

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