

# CBS overexpression can lower homocysteine in mammals

L Wang, K Jhee, X Hua, P DiBello, D Jacobsen, and W Kruger, Fox Chase Cancer Center, 333 Cottman Ave, Philadelphia, PA 19111 USA



**Using the Biochrom 30 Amino Acid Analyzer, homocysteine and other amino acid levels were monitored from a transgenic mouse in which CBS activity could be modulated. Data is presented showing that elevating CBS activity is an effective method to lower plasma homocysteine levels**

Elevated plasma total homocysteine (tHcy) is an important risk factor for the development of vascular disease in humans. One of the metabolic fates of homocysteine is transsulfuration to form cystathionine. The transsulfuration reaction is performed by the enzyme cystathionine  $\beta$ -synthase (CBS). Here we show that CBS overexpression can lower plasma homocysteine levels in mammals.

## Experimental conditions

A transgenic mouse containing the human CBS cDNA under control of the zinc metallothionein promoter (tgCBS) was generated to modulate CBS activity. The expression of the MT promoter is regulated by the addition of zinc to drinking water. The MT promoter is expressed at high levels in the liver and kidney.

## Results

### Transgene characterization

CBS activity was measured by cystathionine formation in the liver and kidney of tgCBS animals and non-transgenic littermates using a Biochrom 30 Amino Acid Analyzer. In the absence of zinc, there was no difference in CBS activity levels between transgenic and non-transgenic animals in either the liver or kidney. With the addition of zinc to the drinking water, in the liver the mean activity of the transgenic animals was 2.2 fold higher than the non-transgenic control animals. In the kidney, there was 3.5-fold elevation in CBS activity between transgenic and non-transgenic animals.

### Effects of CBS Overexpression

Serum was collected from transgenic and nontransgenic littermates fed on standard mouse chow and zinc-containing water. All animals were then shifted to normal water for 2 weeks and rebled. Serum was analysed from tHcy, cysteine, and cysteinyl-glycine. When transgenic mice were put on zinc-containing water, the level of tHcy decreased 45%. Both serum cysteine and cysteinyl-glycine were lower in zinc-treated transgenic animals compared with identically treated control animals. Results show CBS overexpression can significantly lower serum homocysteine.

Amino acid pools in total liver homogenates were measured using a Biochrom 30 Amino Acid Analyzer. Present at detectable levels were threonine, serine, asparagine, glutamine, glutamic acid, glycine, alanine, valine, homocysteine, methionine, cystathionine,

isoleucine, leucine, tyrosine, phenylalanine, lysine, histidine, cysteine, ornithine, glutathione, urea, taurine, phosphoserine, and  $\alpha$ -butyric acid

### Functional complementation of mouse *cbs*

Homozygotes with a knockout allele of CBS (*CBS<sup>tm1Unc</sup>*) have severe homocysteinemia, growth retardation, and rarely survive >6 weeks. To determine whether the tgCBS transgene could rescue these effects, tgCBS animals were crossed to *CBS<sup>tm1Unc</sup>/CBS<sup>tm1Unc</sup>*. The TgCBS *CBS/CBS<sup>tm1Unc</sup>* offspring were put on zinc-containing water and backcrossed to *CBS/CBS<sup>tm1Unc</sup>*. Results demonstrated that the transgene was able to rescue lethality as approximately 1/8th of the offspring were homozygous for *CBS<sup>tm1Unc</sup>* and hemizygous for the transgene.

Levels of tHcy in TgCBS *CBS/CBS<sup>tm1Unc</sup>* were measured using a Biochrom 30 Amino Acid Analyzer in the absence and presence of zinc. Elevated tHcy was observed when mice were switched from zinc-containing to non-zinc containing water, demonstrating that when CBS expression is highest, tHcy levels are lowest.

### Conclusions

- CBS-overexpressing animals have reduced levels of liver homocysteine and elevated levels of cystathionine showing that elevating CBS activity is an effective method to lower plasma homocysteine levels.

- Significant changes in amino acid levels were in those not directly related to methionine or cysteine metabolic pathways, showing that overexpression of a single amino acid biosynthetic pathway can have global consequences.

- Expression of tg-CBS rescued the severe hyperhomocysteinemia and neonatal lethality of *Cbs* deletion animals, showing that expression of human CBS can functionally complement the deletion of mouse *hbs*.

Data excerpted from L Wang, K Jhee, X Hua, P DiBello, D Jacobsen, and W Kruger. **Modulation of Cystathionine  $\beta$ -Synthase Level Regulates Total Serum Homocysteine in Mice** *Circ. Res.*, May 2004; 94: 1318–1324. © 2004 American Heart Assoc. Inc.