The investigation of nicotinamide nucleotide transhydrogenase gene in glucose and energy metabolism. (PhD dissertation)

ABSTRACT

The C57BL/6J (B6J) mice are the most widely utilized strain in a metabolic research of glucose intolerance, which contain a deletion mutation in *Nnt* gene (exons 7- 11). *Nnt* gene plays important role in glucose and energy metabolism. It has been reported that the mutant *Nnt* gene resulted in reduced insulin secretion and increased the mitochondrial reactive oxygen species (ROS) in B6J mice. In this study, the blood glucose level has been comparatively analysed in B6J and Kunming (Km) mice, which contain the intact *Nnt* gene. Also, the genetic association has been studied between the *Nnt* gene and the traits of growth and feed efficiency. The results showed as follows;

1. *Nnt* evolution analysis showed that *Nnt* mRNA of *Mus musculus* was closed to *Rattus norvegicus* in coding regions (cds). *Nnt* mRNA evolution in *Danio rerio* was closed to *Callorhinchus milii.* Whereas, *Nnt* mRNA of *Gallus gallus* was not much closed to *Xenopus tropicalis* and the same *Canis lupus familiaris* to *Sus scrofa.* However, *Nnt* mRNA is closely evolution to each 15 species.
2. The blood glucose levels were declined regularly after feeding and reached to the lowest level at 24hr of fasting in both strains. Moreover, the blood glucose level was significantly higher in B6J mice than that in Km mice at 0hr (Just after feeding), 2hr and 10hr of fasting. While, the blood glucose level was significantly higher in Km mice than that in B6J mice at 24hr of fasting.
3. A B6J×Km F2- population (N= 342) was constructed in this study. The variation of glucose level between 0 and 12 hr of fasting of *Nnt* −/− genotype (*Nnt* mutant homozygous) mice was significantly higher than that of *Nnt* +/+ genotype (*Nnt* intact homozygous) mice in the F2 population ($P < 0.05$). While, the *Nnt* +/− genotype has no difference with *Nnt* −/− or *Nnt* +/+ genotype mice.
4. There were significant differences between body weights of B6J and Km mice at 5 weeks of age. While, no significant differences were observed between B6J and Km body weights at 3 weeks of age.
5. The correlation between *Nnt* gene and metabolism, growth and feed conversion efficiency

ratio (FCR) traits of mice were performed using the F2 population. The results showed that *Nnt* gene was significantly associated with the body weight of 3 weeks (IBW) ($P<0.01$) and 5 weeks (FBW) ($P<0.05$) as well as average metabolic body weight (AMBW) ($P<0.01$), but not associated with average daily feed intake (AFI), average daily gain (ADG) and FCR traits.

6. The blood glucose level was not much different between the high- FCR and low- FCR mice.
7. The *Nnt* gene was highly expressed at intestine, heart, lung and skeletal muscle tissues. The expression levels of *Nnt*, *Glut-4* and *Igf-1* genes in liver tissues were not significantly different between high- FCR and low- FCR mice.
8. The expression levels of *Glut-1* and *Ucp-2* genes were significantly higher in the high- FCR mice than that in the low- FCR mice. While, the expression levels of *Akt-1*, *Glut-2* and *Irs-1* genes were significantly higher in the low- FCR mice than that in the high- FCR mice ($P<0.05$).
9. *Nnt* gene was expressed in C2C12 cells, and the expression level was not much different during differentiation.
10. The dual- luciferase reporter assay indicated that the *Nnt* gene could be targeted by miR-221 and miR-222 in C2C12 myoblast cells.

These results indicate that *Nnt* gene is related to glucose stability, basal energy metabolism and growth, but not to feed efficiency traits in mice. This study offered novel evidences to the role of *Nnt* gene on metabolism and growth. Further, the *Nnt* gene could be regulated by miR-221 and miR-222.

Keywords: *Nnt*; blood glucose level; average daily feed intake; feed conversion ratio; mice