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Introduction

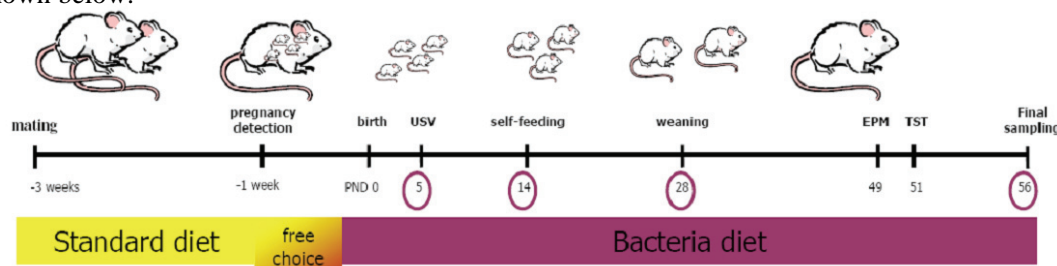
Anxiety disorders are affecting a great number of people in the world. Animal models for trait anxiety offer a promising tool to identify biomarkers for this phenotype. The proteomes of mouse models established for high (HAB), low (LAB) or normal (NAB) anxiety-related behavior were analyzed to discover protein biomarkers.

The ¹⁵N metabolic labeling approach can offer accurate quantification results and avoids the introduction of artificial variations during sample preparation. Animals are fed with either ¹⁴N or ¹⁵N enriched diets. In order to achieve high ¹⁵N incorporation rates in a relative short time, mice are fed *in utero* with stable isotope enriched diets. These diets are made up of nitrogen-free nutrients and a controlled protein source. Initial experiments indicated that labeling with *Spirulina* based diets *in utero* resulted in developmental defects and death of mouse pups 2-3 weeks after birth. Diets using *Ralstonia eutropha* bacterial protein source, however, did not have any detrimental effects.

The established procedure using *Ralstonia eutropha* bacteria allows efficient ¹⁵N metabolic labeling without the need to feed the label over several mouse generations. Accurate relative protein quantitation by mass spectrometry is feasible. A number of regulated proteins that represent candidate biomarkers for trait anxiety have been discovered using this method.

Animal labeling

CD1 mice have been selectively and bidirectionally bred for either high anxiety (HAB) or low anxiety (LAB) related behavior using the elevated plus-maze (EPM). They were fed with ¹⁴N or ¹⁵N enriched diets using the protocol shown below.



Results

¹⁵N incorporation rates at different post natal days (PND)

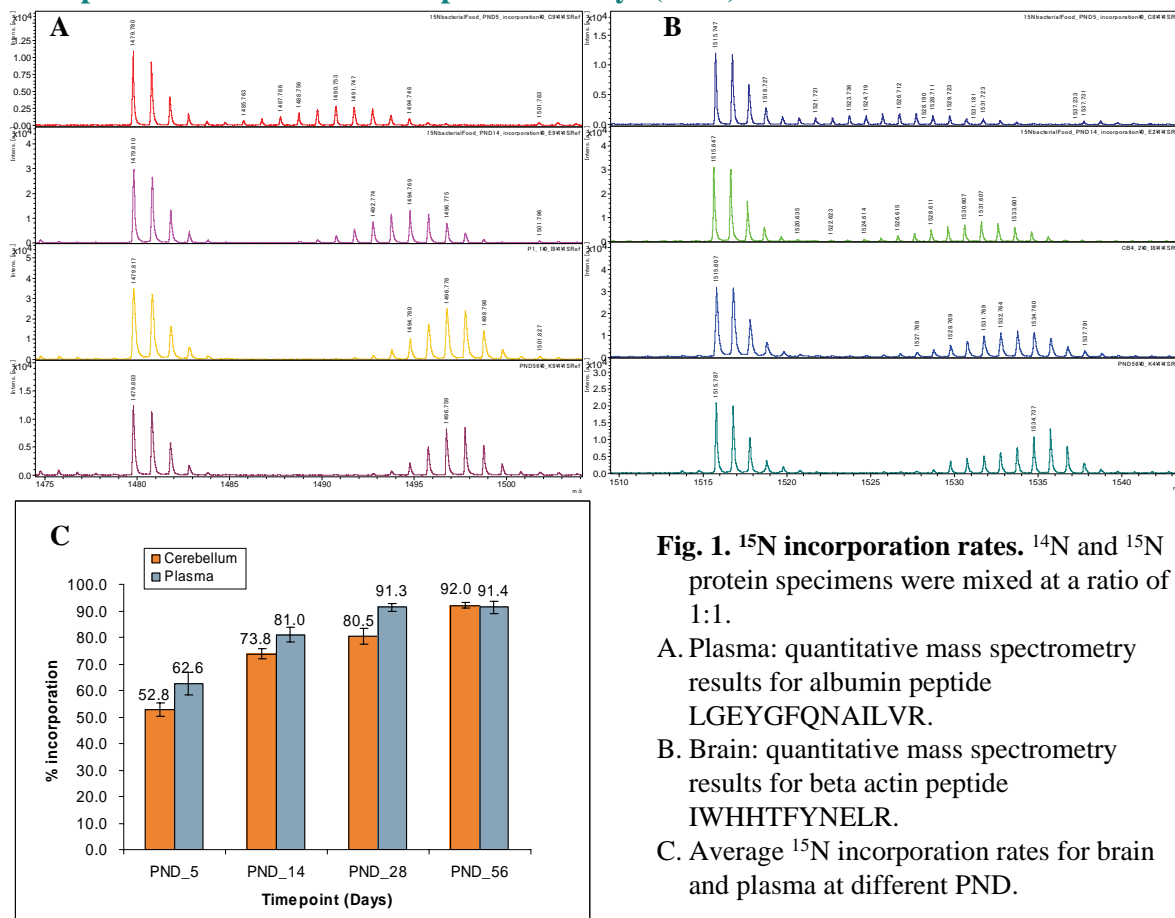


Fig. 1. ¹⁵N incorporation rates. ¹⁴N and ¹⁵N protein specimens were mixed at a ratio of 1:1. A. Plasma: quantitative mass spectrometry results for albumin peptide LGEYGFQNAIVLR. B. Brain: quantitative mass spectrometry results for beta actin peptide IWHHTFYNELR. C. Average ¹⁵N incorporation rates for brain and plasma at different PND.

Behavioral test

Elevated Plus Maze (EPM) tests were carried out for bacterial diet-fed mice to study the effect on the animals' anxiety-related behavior. No differences compared to mice fed with a standard diet were observed.

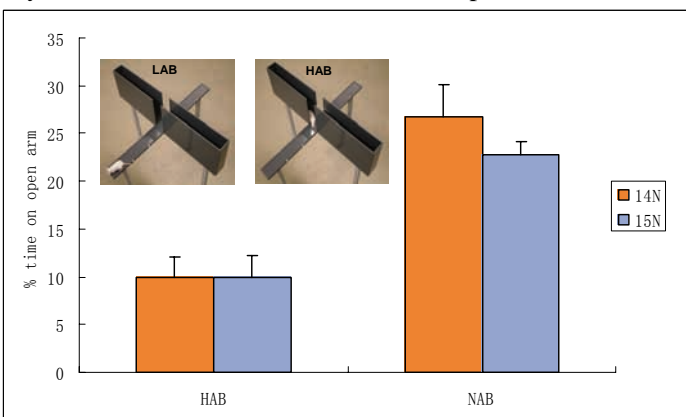
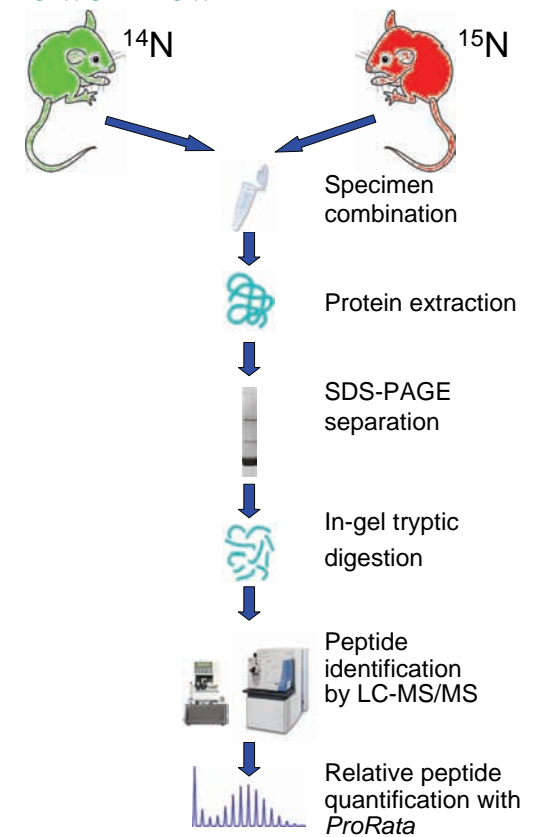


Fig. 2. EPM results for HAB and NAB mice. No difference in time spent on the open arm was observed between animals fed with standard and bacterial diets.

Proteomic workflow



Protein expression results

Table 1. Glyoxalase 1 regulation in ¹⁵N-HAB and ¹⁴N-LAB red blood cells

Sequence	Log ₂ R(¹⁴ N/ ¹⁵ N)	Log ₂ SNR
DFLLQQTMLR	4.3	3.7
DFLLQQTMLR	4.5	3.3
RFEELGVK	3.2	4.8
VLGLTLLQK	3.3	5.3

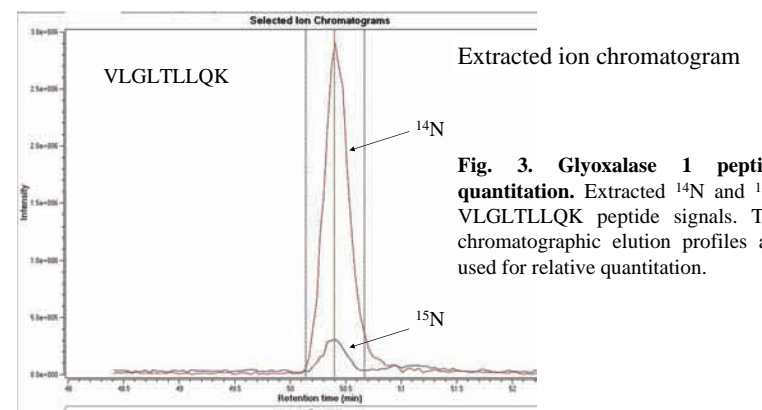


Fig. 3. Glyoxalase 1 peptide quantitation. Extracted ¹⁴N and ¹⁵N VLGLTLLQK peptide signals. The chromatographic elution profiles are used for relative quantitation.

Discussion

Our approach demonstrates that mice can be successfully labeled by feeding them with ¹⁵N enriched bacteria diet.

Mice do not have to be fed the bacterial diet over several generations to achieve high ¹⁵N incorporation rates.

The mice showed no change in their anxiety-related phenotype.

Our quantitative proteomic approach can identify protein markers that are differentially expressed in CD1 mice with different genetic predispositions to anxiety-related behavior.

References

- Krömer, S.A. et al, Identification of glyoxalase-I as a protein marker in a mouse model of extremes in trait anxiety. J. Neurosci., 2005, 25, 4375-4384.
- Ditzen, C. et al, Protein biomarkers in a mouse model of extremes in trait anxiety. Mol. Cell. Proteomics, 2006, 5, 1914-1920.