# Effect of maternal glucoraphanin intake on chronic inflammation in the livers and hypothalamic tissues of high fructose-diet-fed rat offspring exposed to maternal undernutrition.

Anishma Karmacharya*

Graduate School of Health Sciences, Aomori University of Health and Welfare

**Key words** ①Glucoraphanin, ②Fructose, ③Inflammation, ④AMPK, ⑤Maternal undernutrition

## Ⅰ. Background

The fetal and neonatal environment is associated with subsequent development of diseases in adulthood. Maternal undernutrition during pregnancy and lactation can cause dyslipidemia, hyperglycemia which can affect different organs and cause metabolic disorders such as chronic liver disease, Type 2 diabetes in offspring during their adulthood (1). A high-fructose diet (HFD) increases glucose intolerance, insulin resistance, dyslipidemia, and liver inflammation (2).

AMP-activated protein kinase (AMPK) plays a major role in regulating glucose and lipid metabolism in various tissues and organs, including the liver and skeletal muscle.

Glucoraphanin (GR) derived from broccoli, is a water soluble, relatively inert precursor of sulforaphane; the reactive isothiocyanate which has shown to be effective in different metabolic disorders such obesity, insulin resistance, and non-alcoholic fatty liver disease (NAFLD) (3). However, less is known about the effect of GR intake during lactation on the insulin resistance and liver inflammation in the adult rat offspring programmed by maternal protein restriction.

## Ⅱ. Objectives

The aim of the present study was to explore the effect of maternal intake of GR-containing broccoli powder (BP) during lactation on inflammation and dysfunction of autophagy in livers and hypothalamic tissues of high fructose-diet-fed adult rat offspring from dams fed on normal- or low- protein diets during pregnancy and lactation.

## Ⅲ. Materials and Methods

The Animal Research Committee, Aomori University of Health and Welfare, approved this study and all experimental procedures were performed in accordance with the Institutional Guidelines for Animal Experimentation (Permission number: 21008). Pregnant rats received diets containing 20% (Normal Protein; NP) or 8% (Low Protein; LP) casein. While 0 or 0.74% BP containing NP diets (NP/NP or NP/ NP+BP) in Experiment (Expt.) 1 and 0 or 0.74% BP containing LP diets (LP/LP or LP/LP+BP in Expt. 2 were provided during lactation. At weaning, pups that received a diet of distilled water (W) or 10% fructose solution (Fr) were divided into six groups: NP/NP/W, NP/NP/Fr, NP/NPBP/Fr in Expt. 1 and LP/LP/W, LP/LP/Fr, LP/ LPBP /Fr in Expt. 2. At week 13 after treatment, male offsprings were weighed, and blood samples were taken. The samples that we analyzed have already collected in 2023. The plasma glucose (Glc) and insulin levels were measured. Homeostatic model assessment of insulin resistance (HOMA-IR), a biomarker of insulin sensitivity, was performed according to the equation (4). Paraformaldehyde-fixed livers and hypothalamic tissues were embedded in paraffin and cross-sections were prepared. Then, their sections were stained for macrophages by incubating an anti-CD68 antibody, and the number of macrophages in the livers were counted. However, the number of microglia that serve as brain macrophages has not been determined, because the staining methods are

*連絡先：〒030-8505　青森市浜館間瀬 58-1　E-mail: 2293001@ms.auhw.ac.jp

still being established. To examine protein expression of AMPK and NFκB p65 in the western blot analysis of livers was carried out. Because the western blot analysis for hypothalamic tissues are still being established, the protein expression levels in the hypothalamic tissues have not been examined.

## IV. Results and Discussion

### 1. Effect of BP intake during lactation on plasma insulin and HOMA-IR index

In Expt. 1, plasma Glc levels and HOMA-IR values were significantly higher in the NP/NP/Fr and NP/NPBP/Fr than in the NP/NP/W. However, there was no significant difference between the NP/NP/Fr and NP/NPBP/Fr. No significant differences in the plasma insulin levels were found among the three groups. In Expt. 2, plasma Glc levels in the LP/LP/Fr and LP/LPBP/Fr were increased significantly compared to the LP/LP/W. No significant differences were observed between the LP/LP/Fr and LP/LPBP/Fr. Insulin levels in the LP/LP/Fr were significantly higher than those in the LP/LP/W. Conversely, the levels were significantly lower in the LP/LPBP/Fr than in the LP/LP/Fr. Similarly, the HOMA-IR values in the LP/LPBP/Fr were significantly lower than in the LP/LP/Fr.

### 2. Effect of BP intake during lactation on macrophages infiltration

The number of macrophages in the NP/NP/Fr was significantly increased compared to the NP/NP/W; however, there was no significant difference between the NP/NP/Fr and NP/NPBP/Fr in Expt.1. In Expt. 2, the macrophage number in the LP/LP/Fr was significantly higher than that in the LP/LP/W. Conversely, the number in the LP/LPBP/Fr was significantly lower than in the LP/LP/Fr, suggesting that BP intake during lactation may suppress the macrophage infiltration in the liver of HFD-fed adult offspring programmed by maternal protein restriction.

### 3. Effect of BP intake during BP on NFκB p65 protein expression

NFκB p65 protein expression levels were significantly higher in the NP/NP/Fr and NP/NPBP/Fr than the NP/NP/W, but not significantly different between the NP/NPBP/Fr and NP/NP/Fr. In Expt. 2, NFκB p65 protein expression levels were significantly higher in the LP/LP/Fr than the LP/LP/W. Conversely, these levels were significantly lower in the LP/LPBP/Fr than the LP/LP/Fr.

## V. Conclusion

We showed that maternal intake of BP ameliorated insulin resistance and liver inflammation via the upregulation of AMPK activation and/or the downregulation of NFκB activation of HFD-fed adult offspring programmed by maternal protein restriction.

## VI. References

1. Lillycrop KA et al. J Nutr. 2005; 135: 1382.
2. Castro M et al. Life Sci. 2015; 137: 1.
3. Zhang DM et al. Nutrients. 2017; 9: 335.
4. Okita K et al. Endocr J. 2013; 60: 283.

## VII. Presentation

・Karmacharya A, et al. Effect of maternal broccoli powder intake on autophagy flux in the livers of high fructose-fed male rat offspring from protein restricted mothers. 第 77 回日本栄養食糧学会大会, 2023 年 5 月, 札幌市

・Karmacharya A, et al. Effect of maternal glucoraphanin intake on lipid and glucose metabolism in high fructose-fed male rat offspring exposed to maternal protein restriction. 2023 年度青森県保健医療福祉研究発表会, 2023 年 12 月，青森市