Effect of maternal broccoli powder intake on autophagy flux and epigenetic enzymes in the livers of high fructose-fed male rat offspring exposed to maternal protein restriction

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I. Background

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) is a major contributor to the increased glucose intolerance, insulin resistance, dyslipidemia, and liver inflammation[2].

Glucoraphanin (Gr) derived from broccoli, is a water soluble, relatively inert precursor of sulforaphane (SFN); 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].

Autophagy is the vital process that clears out protein aggregates and damaged organelles. In contrast, dysregulated autophagy is associated with onset of insulin resistance. Broccoli derived SFN has reported to trigger lipophagy, a form of selective autophagy via AMP-active protein kinase (AMPK)/mechanistic target of rapamycin (mTOR) pathway. However, less is known about the effect of maternal broccoli intake during lactation in the autophagy flux and epigenetic modulation in adult rat offspring.

II. Objectives

In this study, we investigated that effect of maternal intake of Gr-containing broccoli powder (BP) during lactation on hepatic AMPK/mTOR pathway and autophagy flux in HFD-fed male offspring programmed by maternal protein restriction. In addition, we assessed the epigenetic modulatory action of BP diet.

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 Wistar rats received diets containing 20% (NP) or 8% (LP) casein and 0 or 0.74% BP containing NP diets (NP/NP or NP/NPBP) in experiment (Expt.) 1 and 0 or 0.74% BP containing LP diets (LP/LP or LP/LPBP) in Expt. 2 during lactation. At weaning, male offspring 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. The samples that we analyzed in this study have already collected in 2023. Paraformaldehyde-fixed livers 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. At week 13, AMPK and mTOR phosphorylation, and autophagy related protein: microtubule-associated protein 1 light chain 3 beta (LC3B), mRNA of DNA methyltransferases (DNMTs) 1 and DNMT3a were examined.

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IV. Results and Discussion

1. Effect of BP intake during lactation on macrophages infiltration and NFκB p65 protein expression

As we have demonstrated in the report, “Jisseki Houkoku-syo” in 2023, the macrophages number in the NP/NP/Fr was significantly increased compared to the NP/NP/W, whereas there was no significant difference between the NP/NP/Fr and NP/NPBP/Fr in Expt.1. In Expt. 2, the macrophage number and NFκB p65 protein expression levels were in the LP/LP/Fr were significantly higher than those in the LP/LP/W. Conversely, the number and the expression levels in the LP/LPBP/Fr was significantly lower than in the LP/LP/Fr, suggesting that BP intake during lactation may suppress the inflammation in the liver of HFD-fed adult male offspring programmed by maternal protein restriction.

2. Effect of BP intake during lactation on protein expression and phosphorylated AMPK and mTOR

In Expt. 2, the phosphorylated AMPK (p-AMPK) levels were significantly higher in the LP/LPBP/Fr than the LP/LP/Fr. The phosphorylated mTOR (p-mTOR) levels were significantly higher in the LP/LP/Fr than the LP/LP/W. Conversely, the levels were lower in the LP/LPBP/Fr than the LP/LP/Fr. In Expt. 1, no changes in p-AMPK levels across different groups was found. The p-mTOR levels were significantly higher in the NP/NP/Fr than the NP/NP/W and were lower in the NP/NPBP/Fr than the NP/NP/Fr.

3. Effect of BP intake during lactation on autophagy markers

In Expt. 2, the levels of LC3BII were lower in the LP/LP/Fr than those of the LP/LP/W while higher in the LP/LPBP/Fr than in the LP/LP/Fr. In Expt. 1, no significant changes were found in the level of the autophagy markers across different groups. It has been reported that mTOR activity negatively regulates autophagy flux. Therefore, BP intake during lactation may contribute to the upregulation of autophagy flux through the mTOR pathway in the livers of HFD-fed adult offspring programmed by maternal low protein diet.

4. Effect of BP intake during lactation on epigenetic enzymes; DNMT1 and DNMT3a

In Expt. 2, the levels of both DNMT1 and DNMT3a increased significantly in the LP/LP/Fr group compared to the LP/LP/W. Conversely, DNMT1 and DNMT3a levels were significantly lower in the LP/LPBP/Fr than the LP/LP/Fr, respectively. These results suggest that BP intake may modulate epigenetic regulation in the livers of HFD-fed offspring. In Expt. 1, no significant differences in the DNMT1 and DNMT3a mRNA expression levels were found.

V. Conclusion

In conclusion, maternal BP intake during lactation may cause long-term alterations in the autophagy flux in the livers of HFD-fed adult male offspring from protein-restricted mother. This result may be associated with the epigenetic modulation.

VI. References

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