Investigation into the effects of long-term enteral nutrition on gut microbiota and age-related changes

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Abstract

The aim of this study was to investigate the effects of long-term enteral nutrition on gut microbiota and aging in individuals with severe motor and intellectual disabilities (SMID). In Japan, enteral nutrition therapy is widely implemented for elderly individuals with difficulties in oral intake. While effective for short-term nutritional improvement, its long-term effects remain unclear. This study hypothesized that prolonged enteral nutrition disrupts the gut microbiota and accelerates aging. A total of 47 individuals, including SMID patients and healthy controls, participated in the study. Gut microbiota diversity and short-chain fatty acid (SCFA) concentrations were analyzed using 16S rRNA sequencing and LC-MS. The results revealed that the SMID group exhibited significantly reduced gut microbiota diversity and lower SCFA concentrations compared to the healthy controls. Furthermore, analysis based on nutritional intake methods showed significant differences in formic acid and succinic acid concentrations between enteral nutrition and oral intake groups. Biological age, as assessed in this study, was higher in the enteral nutrition group than in the oral intake group. These findings suggest that long-term enteral nutrition may influence gut microbiota composition and contribute to accelerated aging.

1. Aim of Research

The objective of this study is to elucidate the effects of long-term enteral nutrition on gut microbiota and aging. In Japan, enteral nutrition therapy is widely administered to elderly individuals with difficulties in oral intake. While effective in improving short-term nutritional status, its long-term effects remain insufficiently understood. Notably, the physical properties of enteral nutrition differ substantially from those of oral intake, inevitably impacting intestinal metabolism and gut microbiota. Moreover, the effects of prolonged enteral nutrition on aging remain an unexplored area. In recent years, the concept of "biological age," which goes beyond chronological age, has garnered attention in aging research. Biological age is estimated using methods based on DNA methylation and gene expression, enabling more precise assessments of individual aging status. Furthermore, biological age is known to be influenced by lifestyle factors such as diet and exercise. However, the impact of enteral nutrition on biological age has not been clarified.

Against this background, this study aims to test the hypothesis that long-term enteral nutrition disrupts gut microbiota and accelerates biological aging. The study focuses on individuals with severe motor and intellectual disabilities (SMID) who have been hospitalized since infancy, with analyses conducted to investigate these effects.

2. Method of Research & Progression

2-1 Subjects and Nutritional Assessment

This study included 47 individuals with SMID and 47 healthy controls. Among the SMID participants, 18 were on oral intake, while 29 were on enteral nutrition. Of the 29 receiving enteral nutrition, 10 were on

natural nutrient-rich liquid diets, and 19 were on artificial nutrient-rich liquid diets. Dietary records, including the type and amount of enteral nutrition, were collected for two weeks before and after sample collection to evaluate nutritional status. Nutritional assessments for the healthy control group were conducted using the BDHQ dietary questionnaire.

2-2 Gut Microbiota Analysis

Stool samples were collected using commercial kits and stored at −80°C. After all samples were collected, DNA was extracted from the stool specimens, and quality assessment was performed. 16S rRNA sequencing was conducted using a high-speed sequencer to analyze the composition and ratios of gut microbiota, as well as to investigate their functional characteristics. Additionally, short-chain fatty acid (SCFA) analysis was performed using liquid chromatography-mass spectrometry (LC-MS).

2-3 Gene Expression Analysis

RNA was extracted from peripheral blood samples using the PAXgene Blood RNA Kit and stored at −80°C. After completing the RNA collection for all samples, RNA sequencing was conducted to identify differentially expressed genes and analyze their associated functions.

2-4 Biological Age Analysis

Biological age was estimated using the RNAAgeCalc tool, which operates within the R programming environment. Normalized gene expression data obtained in Section 2-3 were used as input for calculating biological age.

3. Results of Research

3-1 Characteristics of Participants and Nutritional Assessment

The group of individuals with SMID had an average age of 15 years (range: 2–30 years), with 46.8% being male, and an average BMI of 13.8. In contrast, the healthy control group had an average age of 15 years (range: 3–30 years), with 51.1% being male, and an average BMI of 18.8. The Bristol Stool Scale scores, indicating stool condition, were 4.7 for the SMID group and 3.2 for the control group.

In terms of nutritional assessment, the SMID group had an average intake of 1192 kcal of total energy, 38 g/1000 kcal of protein, 30 g/1000 kcal of fat, 142 g/1000 kcal of carbohydrates, and 11 g/1000 kcal of dietary fiber. For the healthy controls, the average intake was 1789 kcal of total energy, 36 g/1000 kcal of protein, 33 g/1000 kcal of fat, 134 g/1000 kcal of carbohydrates, and 6 g/1000 kcal of dietary fiber. Although the total energy intake in the SMID group was significantly lower than that in the control group, the ratios of major nutrients were not markedly different.

3-2 Gut Microbiota Analysis

Alpha diversity analysis revealed that the gut microbiota diversity was significantly lower in the SMID group compared to the control group. Beta diversity analysis also showed distinct differences in the gut microbiota composition between the two groups.

Regarding bacterial composition, the SMID group exhibited a higher prevalence of Bacteroides and a lower prevalence of Bifidobacterium compared to the controls. Additionally, bacteria associated with loose stools, such as Klebsiella and Escherichia coli, were detected in the SMID group.

SCFAs, which are organic acids produced through gut microbiota metabolism and are considered important for maintaining intestinal health, were analyzed. The concentrations of acetate and propionate were significantly lower in the SMID group. Furthermore, a comparison between the oral intake and enteral nutrition (artificial nutrient-rich liquid diet) groups within the SMID population showed significant differences in the concentrations of formic acid, succinic acid, and lactic acid.

3-3 Biological Age Analysis

The SMID group showed notable differences in gut microbiota and SCFA production

compared to the control group. Within the SMID group, significant differences were observed between the oral intake and enteral nutrition (artificial nutrient-rich liquid diet) groups, prompting an investigation into the effects of nutritional intake methods on aging.

Biological age was estimated using the RNAAgeCalc tool based on gene expression data. The results showed that the oral intake group had an average chronological age of 20 years and an average biological age of 31 years. In comparison, the enteral nutrition (artificial nutrient-rich liquid diet) group had an average chronological age of 20 years and an average biological age of 32 years. These findings suggest that biological aging may progress more rapidly in the enteral nutrition group compared to the oral intake group.

4. Future Area to Take Note of, and Going Forward

This study focused on individuals with SMID who were hospitalized long-term, aiming to minimize the influence of environmental factors. However, while the primary cause of SMID is often complications during childbirth, the underlying conditions vary widely. This diversity may have influenced the study's results and cannot be entirely ruled out.

Regarding biological age analysis, recent advancements have seen widespread use of methods based on DNA methylation for estimating biological age. Moving forward, incorporating DNA methylation-based analyses will allow for a more comprehensive evaluation of biological aging.

Additionally, during the course of this study, the principal investigator relocated to a new institution, necessitating the establishment of a new laboratory and the resubmission of research protocols to the ethics committee. Addressing these unforeseen circumstances required modifications to certain aspects of the original research methods. The reliability and validity of the resulting data will continue to be rigorously evaluated to ensure the integrity of the study's findings.

5. Means of Official Announcement of Research Results

Although data analysis is still ongoing, the findings of this study are planned to be disseminated through presentations at domestic and international conferences and publications in peer-reviewed international journals. These efforts aim to widely share the research outcomes and contribute to the advancement of related fields.

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