Clinical Nutrition xxx (2016) 1–8

Contents lists available at ScienceDirect

Clinical Nutrition

journal homepage: http://www.elsevier.com/locate/clnu

Original article

# Nutritional assessment using stable isotope ratios of carbon and nitrogen in the scalp hair of geriatric patients who received enteral and parenteral nutrition formulas

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ARTICLE INFO

*Article history:*
Received 7 June 2016
Accepted 17 October 2016

*Keywords:*
Stable isotope ratio of carbon
Stable isotope ratio of nitrogen
Scalp hair
Calorie
Enteral nutrition
Parenteral nutrition

SUMMARY

*Background & aims:* The $\delta^{13}C$ and $\delta^{15}N$ values in the scalp hair of geriatric patients in Japan who received the enteral or parenteral nutrition formula were measured to assess nutritional status.
*Methods:* The relations among $\delta^{13}C$, $\delta^{15}N$, calorie intake, BMI, albumin concentration, total cholesterol (T-CHO) and geriatric nutritional risk index (GNRI) in the patients were investigated. Furthermore, the enrichment of $\delta^{13}C$ and $\delta^{15}N$ from the nutrients to the hair was investigated.
*Results:* The $\delta^{13}C$ values in the hair of patients who received enteral nutrition decreased with decreases in the calories received, while the $\delta^{15}N$ values increased, suggesting malnutrition in some patients with a low calorie intake due to a negative nitrogen balance. The distribution of patients with a low calorie intake (below 20 kcal/kg/day) when $\delta^{13}C$ was plotted against $\delta^{15}N$ differed from that of control subjects, but the distribution of patients with a high calorie intake (above 20 kcal/kg/day) was similar to that of control subjects. No significant differences were observed in BMI, albumin concentration, T-CHO or GNRI between the low and high calorie groups. The enrichment of $\delta^{13}C$ and $\delta^{15}N$ from the enteral nutrients to the hair were inversely correlated with the $\delta^{13}C$ and $\delta^{15}N$ in the enteral nutrients. The enrichment levels of $\delta^{13}C$ and $\delta^{15}N$ tended to be higher and lower, respectively, in the high calorie group. On the other hand, the $\delta^{13}C$ and $\delta^{15}N$ values in the hair of patients who received parenteral nutrition were higher and lower than those in the control subjects and in the patients who received enteral nutrition, respectively, reflecting the higher $\delta^{13}C$ and lower $\delta^{15}N$ contents of the parenteral nutrients.
*Conclusions:* The $\delta^{13}C$ and $\delta^{15}N$ values in the hair of patients who received enteral nutrition may be effective indicators for evaluating the long-term nutritional status of geriatric patients. A calorie intake of 20 kcal/kg/day may be a cut-off value for malnutrition in Japanese geriatric patients receiving enteral nutrition. However, caution is necessary when dealing with patients switching from parental nutrition as parenteral nutrition resulted in different changes in $\delta^{13}C$ and $\delta^{15}N$. The enrichment levels of $\delta^{13}C$ and $\delta^{15}N$ from the enteral nutrients to the hair may be inversely correlated with the $\delta^{13}C$ and $\delta^{15}N$ values of enteral nutrients and vary according to the calorie intake.
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## 1. Introduction

Terrestrial plants following the C3 photosynthesis cycle show significantly depleted $^{13}C$ values (about −26‰) compared to C4 plants (about −13‰) [1], and these form the base of the terrestrial food web (Fig. 1). A large amount of basic human food as well as feed for domestic animals is derived from C3 plants (wheat, barley,

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http://dx.doi.org/10.1016/j.clnu.2016.10.017
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Please cite this article in press as: Hayasaka M, et al., Nutritional assessment using stable isotope ratios of carbon and nitrogen in the scalp hair of geriatric patients who received enteral and parenteral nutrition formulas, Clinical Nutrition (2016), http://dx.doi.org/10.1016/j.clnu.2016.10.017

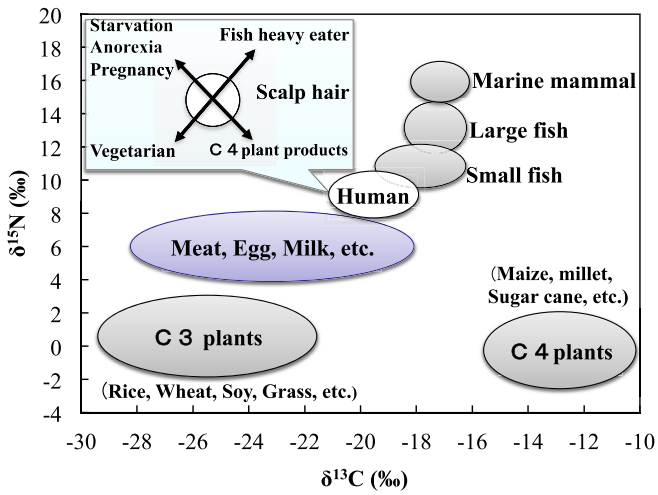


**Fig. 1.** Schema of food web, and foods and illness affecting the stable isotope ratios of carbon and nitrogen in the scalp hair.

soy, potatoes, rice, beans, sugar beet, grass, etc.), although a few species of C4 plants (maize, sugar cane, millet, etc.) are dominant in large regions. Recently, not only sugars and bioethanol but also many amino acids have been produced from C4 plants [2].

The stable isotope ratio of nitrogen ($\delta^{15}N$) is used to estimate the trophic level of a food chain, while the stable isotope ratio of carbon ($\delta^{13}C$) is used to estimate the relative contribution to the diet of potential primary sources [1]. Many researchers have reported high levels of $\delta^{15}N$ in the muscle of marine mammals and predatory fish, reflecting their high trophic positions [3–5] (Fig. 1), and in the scalp hair of heavy fish-eaters and marine mammal-eaters [6,7]. Furthermore, $\delta^{13}C$ and $\delta^{15}N$ in the scalp hair are used to assess nutritional and metabolic status, food supply and as a diagnostic tool [6,8–11], as the scalp hair can be noninvasively sampled and records the long-term history of dietary and physiological conditions in comparison with blood and urine.

BMI as well as serum albumin is widely used as convenient indicator for nutritional access. Recently, the geriatric nutritional risk index (GNRI) [12], which is calculated from the BMI and albumin, has been used to assess the nutritional status of senior patients in Japan [13,14].

Japanese society is aging rapidly and the number of hospitalized senior patients receiving enteral or parenteral nutrition is increasing. In our pilot study [10], we assessed the nutritional status of geriatric patients receiving enteral nutrition using $\delta^{15}N$ and $\delta^{13}C$ values in the scalp hair, and reported that the $\delta^{15}N$ value in the hair increased with decreases in the calorie intake, while the $\delta^{13}C$ value decreased, and that a calorie intake of 20 kcal/kg/day may be a cut-off level for malnutrition. Interestingly, an intake of 20 kcal/kg/day is reported to be the basal metabolic energy of Japanese women at about 80 years [15]. Detailed study is necessary to confirm these noteworthy results. In this study, we investigated the relations among the $\delta^{13}C$ and $\delta^{15}N$ values in the scalp hair, calorie intake, BMI and serum albumin level, and GNRI for patients who received enteral nutrition. Furthermore, the $\delta^{13}C$ and $\delta^{15}N$ values in the hair of the patients who received parenteral nutrition were also investigated.

## 2. Materials and methods

### 2.1. Ethic statement

This research project and associated consent procedures were approved by the Human Research Ethics Committee of the Graduate School of Pharmaceutical Sciences, Health Sciences University of Hokkaido (No. 15P004). Hair donors provided their written informed consent to participate in this study. When the donor was child or very old patient, the informed consent was obtained from a guardian. The principles of the Declaration of Helsinki were taken into consideration for each part of this study.

### 2.2. Sampling of scalp hair

Scalp hair samples from 184 control subjects, living in Hokkaido, Aomori, Miyagi, Iwate and Yamagata Prefectures and the Tokyo Metropolitan area, Japan, were collected between November 2009 and January 2016. As reported previously [7,10], control subjects are defined as donors of scalp hair who ate normal diets and declared to be healthy without any specific disease. We could not collect hair sample from control subjects over 89 years.

Scalp hair samples from 35 patients, living in Hokkaido, Miyagi and Yamagata Prefectures, Japan, who received enteral nutrition formula for at least 3 months (Table 1), or parenteral nutrition formula for at least 1 month (Table 2), were collected between November 2009 and December 2015 as reported previously [10]. Most of the patients receiving the enteral nutrition formula were hospitalized patients of advanced age (late-stage elderly) with little scalp hair. We collected hair sample from the scalp where possible, and did not collect the samples from a particular region and from a specific length from the scalp. Patients who showed a sudden decrease in body weight within at least one month before collection of hair samples or who had inflammation requiring extra energy in the calculation of the calorie requirements shown below were excluded.

The actual calorie intake for the patients was compared with the predicted resting calorie requirement using the Harris-Benedict equation [16] shown below, where W, H and A are weight (kg), height (cm) and age (years), respectively.

$$\text{Men: Energy (kcal/day)} = 66 + 13.7 \times W + 5 \times H - 6.8 \times A$$

$$\text{Women: Energy (kcal/day)} = 665 + 9.6 \times W + 1.8 \times H - 4.7 \times A$$

The R/P ratio shown in Table 1 indicates the ratio of calories received to the predicted calorie requirement, with the active factor being 1.0. In some patients (Table 2), the brand of parenteral nutrition formula previously received was unknown due to their having been transferred from another hospital.

All hair samples were packed in paper or polyethylene bags and stored at room temperature until analysis. The present study included the data for 17 control subjects and 18 patients reported previously [10].

### 2.3. Enteral and parenteral nutrition formulas

Patients received the following enteral nutrition formulas: Liquid Nutrient K-4S (Kewpie Co. Tokyo, Japan), CZ1.5, E–7II and MA-R2.0 (Clinico Co., Ltd. Tokyo, Japan), Isocal Bag 2K, Isocal 2K Neo, and Isocal Plus (Nestle Health Science, Tokyo, Japan), Meibalance® R Green (Meiji Co., Ltd. Tokyo, Japan), Ensure Liquid® (Abbott Japan Co., Ltd. Tokyo, Japan), and HINE E-GEL (Otsuka Pharmaceutical Factory Inc., Tokushima, Japan). Most patients received those formulas by percutaneous endoscopic gastrostomy feeding, and some via nasal intubation.

Patients received the following parenteral nutrition formulas: FULCALIQ™ 1 and FULCALIQ™ 2 (Terumo Co., Tokyo, Japan), NEOPAREN® No 2 Injection and ELNEOPA® No 2 Injection (Otsuka Pharmaceutical Factory Inc., Tokushima, Japan) and PNTWIN® No 1 (Yoshindo Inc., Toyama, Japan). All patients received these formulas

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