

Original Article

Increased Concentrations of Breath Hydrogen Gas in Japanese Centenarians

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Abstract

Purpose: It has been demonstrated that hydrogen gas and hydrogen-rich water may act as a therapeutic antioxidant. Hydrogen gas produced by intestinal fermentation is believed to have some preventive effects on age-related diseases. This study was performed to determine whether intestinal production of hydrogen gas could be associated with longevity.

Methods: The hydrogen gas concentrations in the breath, reflecting the intestinal production of hydrogen gas, were measured by a portable breath hydrogen analyzer in twenty-six centenarians who were local residents (6 men, 20 women; age, 102 (2) years (mean (SD)) and 16 offspring (7 men, 9 women; 70 (5) years) who lived in the same household. For comparison, the breath hydrogen gas concentrations were also measured in 14 healthy controls (8 men, 6 women; 37.3 (17.4) years) and 15 elderly people with type 2 diabetes (4 men, 11 women; 79 (6) years).

Results: The hydrogen concentrations in the centenarians (59.4 (62.6) ppm) were significantly ($p < 0.05$) higher than those in the healthy controls (17.7 (19.6) ppm) and in the elderly diabetic patients (23.2 (19.0) ppm), but there was no significant difference between the centenarians and their offspring, living in the same home (37.8 (27.2) ppm).

Conclusion: Increased intestinal production of hydrogen gas might contribute to longevity in Japanese centenarians, and it is presumably related to the diet and gut microbiota.

KEY WORDS: Breath hydrogen gas, Centenarians, Longevity, Intestinal fermentation, Gut microbiota

Introduction

Hydrogen molecule is composed of two protons and two electrons, and it is the smallest and most highly combustible gas molecule. Hydrogen is generally considered to be physiologically inert, similarly to nitrogen, even under hyperbaric conditions, and it is often used in deep-sea diving applications¹⁾. Dole *et al.*²⁾ reported the therapeutic potential of hydrogen gas, demonstrating that a mixture of 2.5% oxygen and 97.5% hydrogen at a total pressure of 8 atmospheres caused a regression of skin tumors in hairless albino mice. Since Ohsawa *et al.*³⁾ reported that 2-4% hydrogen gas could act as an antioxidant by selectively reducing cytotoxic oxygen radicals, the beneficial effects of hydrogen gas and hydrogen-rich water have been documented in various disease models and human diseases^{1,4)}.

It is intriguing that hydrogen gas produced by colonic fermentation could also act as an antioxidant^{4,5)}, potentially preventing age-related deleterious diseases. There is no source of hydrogen gas for humans other than bacterial metabolism of carbohydrates, and the hydrogen gas is quickly absorbed into the portal circulation and partially excreted by the lungs⁶⁾. Accordingly, hydrogen breath tests are commonly used to reveal functional gastrointestinal disorders, including carbohydrate malabsorption and small intestinal bacterial overgrowth⁷⁾. In the current study, the breath hydrogen gas was measured in centenarians who were thought to have evaded life-threatening diseases to determine whether intestinal production of hydrogen gas could have any association with longevity.

Methods**Subjects**

People aged 100 years and over (centenarians) during the fiscal year 2010 in Matsumoto (Nagano, Japan) (135 people out of 242 thousand citizens) and Shiojiri (Nagano, Japan) (35 people out of 68 thousand citizens) were asked by post whether they would agree to participate in the current study, which was approved by the Ethical Review Board of National Hospital Organization Matsumoto Medical Center. In total, 39 centenarians and/or their offspring gave their written consent to participate in the study by return of post, and we were able to visit 28 of these centenarians in their own homes or nursing homes. After excluding 2 centenarians who were not capable of performing the deep breathing as instructed, 26 centenarians (6 men, 20 women; age, 102 (2) years (mean (SD)); body height, 144 (9) cm; body mass index (BMI), 19.2 (2.2)) were studied. Of these, 1 had been treated for diabetes, 14 had been treated for hypertension, 4 had been treated for heart failure, 3 had been treated for cerebral infarction, and 5 had been treated for dementia. Among the participants, 5 of the centenarians were bedridden.

In the 26 subjects, the breath hydrogen gas concentrations were measured and blood samples were taken between 11:00 and 18:00 and more than 4 hours after breakfast. The breath hydrogen gas concentrations were also measured in 16 offspring (7 men, 9 women; 70 (5) years) who lived with their respective centenarians in the same home. For comparison,

those concentrations were measured in 14 healthy controls (8 men, 6 women; 37 (17) years; 164 (7) cm; BMI, 20.4 (1.8)), and 15 elderly people with type 2 diabetes (4 men, 11 women; 79 (6) years; 153 (10) cm; BMI, 24.1 (3.1)). The HbA1c levels (National Glycohemoglobin Standardization Program) of the patients were 7.7 (1.4)%. Six patients had diabetic retinopathy, and 4 patients had macroproteinuria. Diabetic patients treated with α -glucosidase inhibitors that cause carbohydrate malabsorption⁸⁾ were not included.

The basic clinical characteristics of the four study groups are summarized in **Table 1**. The postprandial serum levels of glucose, total cholesterol, and albumin in 26 centenarians were 4.7 (1.3) mmol/l, 4.42 (0.98) mmol/l, and 3.6 (0.6) g/dl, respectively.

Table 1 Clinical characteristics of four study groups

	Healthy controls	Elderly diabetics	Centenarians	Centenarians' offspring
n	14	15	26	16
Male/Female	8/6	4/11	6/20	7/9
Age (years)	37 (17)	79(6)	102(2)	70(5)
Body mass index	20.4 (1.8)	24.1(3.1)	19.2(2.2)	NA
Comorbidity (n)				
Diabetes	0		1	NA
Hypertension	0	15	14	NA
Heart failure	0	2	4	0
Cerebral infarction	0	1	3	0
Dementia	0	3	5	0

Mean (SD); NA, not assessed; Heart failure, excluding New York Heart Association (NYHA) Class I; Cerebral infarction, excluding silent lacunar infarction.

Hydrogen Gas Measurements

The breath hydrogen gas concentrations (ppm) were measured in a puff of exhaled breath by a portable breath hydrogen analyzer using a SnO₂-based semiconductor-type gas sensor that was sensitive and specific to hydrogen at 0.1 to several hundred ppm (HYDlyzer, TAIYO, Osaka, Japan). The subjects were instructed to take a long, deep breath, and the hydrogen gas concentrations were detected at the end of



Fig. 1. Measurement of the hydrogen gas concentrations in exhaled air

A female centenarian at age 102 who was instructed to take a long, deep breath is shown. The hydrogen gas concentration was detected at the end of the exhale by a portable breath hydrogen analyzer.

the exhaled breath, as shown in **Fig. 1**. The hydrogen gas concentrations were assumed to be equivalent to those in the capillary blood vessels of the lung.

Statistical Analysis

The data are expressed as the mean (SD). Statistical analysis was performed using ANOVA followed by Bonferroni's multiple comparison test, with a significance level at $p < 0.05$. A correlation analysis was performed between two groups.

Results

Variations of Breath Hydrogen Gas Concentrations

The coefficient of variation of triplicate measurements at six different concentrations of hydrogen gas (1.9-145.6 ppm) was 8.5 (3.8)% (**Fig. 2**, left panel). As shown in the right panel of **Fig. 2**, the diurnal variations or intra- and inter-individual variations of the hydrogen gas concentrations were exemplified in three of the healthy controls. Because the inter-individual variations were found to be larger in the post-absorption period after a breakfast meal, the breath hydrogen gas concentrations measured between 11:00 and 18:00, after breakfast (and lunch) and more than 4 hours after breakfast, were assessed in the current study.

Breath Hydrogen Gas Concentrations in Four Study Groups

Fig. 3 shows the hydrogen gas concentrations of expired breath in the four groups of subjects. The hydrogen gas concentrations in the centenarians (59.4 (62.6) ppm) were significantly ($p < 0.05$) higher than those in the healthy controls (17.7 (19.6) ppm) and in elderly diabetic patients (23.2 (19.0) ppm), but not significantly different from those in their offspring (37.8 (27.2) ppm). When statistical analysis was performed by using log-transformed data, there was a significant difference ($p < 0.01$, unpaired t test) between the healthy controls (1.01 (0.53)) and the centenarians (1.44 (0.66)) or between the healthy controls and the centenarians' offspring (1.42 (0.44)). However, ANOVA in the four groups (1.21 (0.43) for the elderly diabetic patients) showed no significant difference ($p = 0.168$).

As shown in **Fig. 4**, there was not a significant correlation between the breath hydrogen gas concentrations in 16 centenarians and those in their offspring living in the same homes ($r = 0.16$, $p = 0.56$). However, when two centenarians (open circles in Figure 3) who showed distinctly high concentrations of breath hydrogen gas were excluded, the correlation between the two groups became significant ($r = 0.55$, $p < 0.05$).

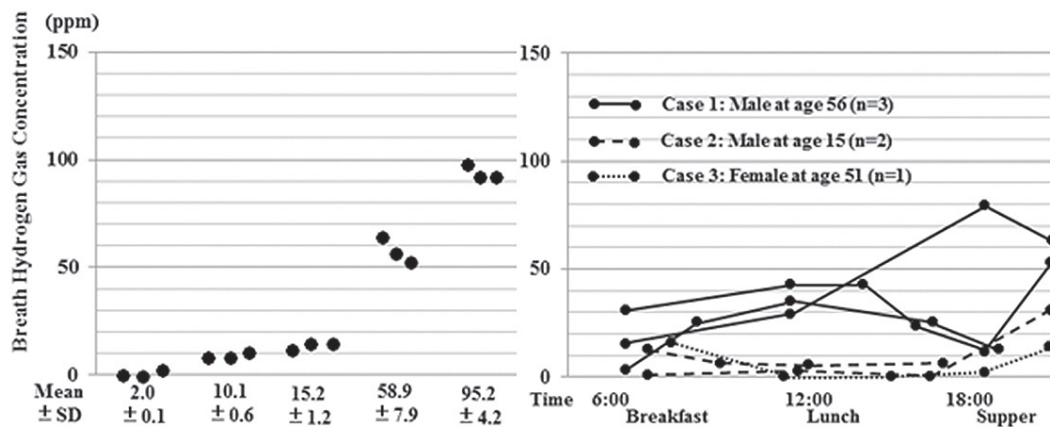


Fig. 2. Variations of the hydrogen gas concentrations in exhaled air
 Left panel: triplicate measurements at six different concentrations of hydrogen gas are shown. The mean (SD) of the coefficient of variation was 8.5 (3.8)%. Right panel: diurnal variations or intra- and inter-individual variations of hydrogen gas concentrations in three cases of healthy controls are shown.

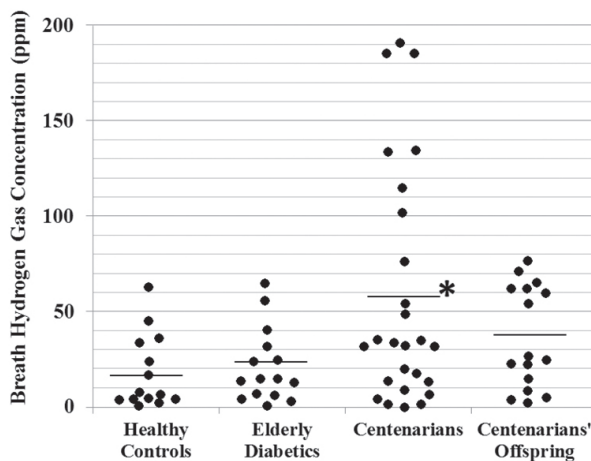


Fig. 3. Breath hydrogen gas concentrations in four groups of participants
 Each dot denotes the hydrogen gas concentration between 11:00 and 18:00, after breakfast (and lunch) and more than four hours after breakfast. The mean concentration (short line) in the centenarians was higher than that in the other groups. * $p < 0.05$ vs. controls and diabetics by ANOVA followed by Bonferroni's test.

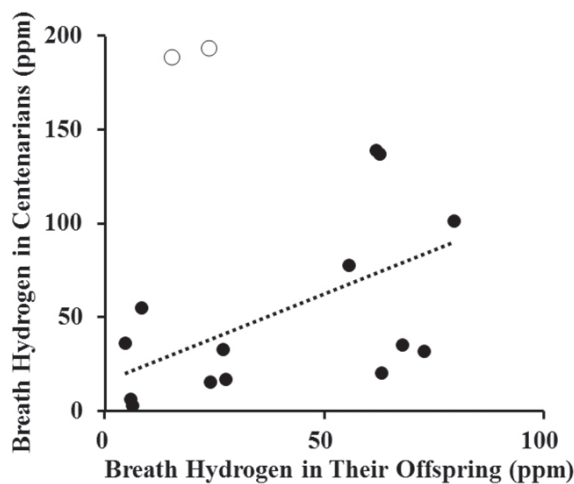


Fig. 4. Breath hydrogen gas in centenarians and their offspring
 There was not a significant correlation between the breath hydrogen gas concentrations in 16 centenarians and those in their offspring living in the same home ($r = 0.16, p = 0.56$). When two cases (open circles) in which the centenarians showed distinctly high concentrations of breath hydrogen gas were excluded, the correlation between the two groups became significant ($r = 0.55, p < 0.05$).

Discussion

In this study, the hydrogen gas concentrations in the breath of Japanese centenarians who were lean but not malnourished were found to be higher than those of elderly people with diabetes and relatively young healthy people. Therefore, it is presumed that the centenarians had increased hydrogen gas production in the intestines, depending on the presence of undigested carbohydrates and hydrogen-producing bacteria, which was affected by environmental conditions and the genetic background^{7,9,10}. This conclusion is supported by the finding that there was a tendency of a correlation between the breath hydrogen gas concentrations of centenarians and their offspring living in the same homes. To my best knowledge, this is the first report regarding breath hydrogen gas in centenarians.

Recently, the beneficial effects of hydrogen-rich water have been documented in human diseases, such as diabetes¹¹, metabolic syndrome¹², cerebral infarction¹³, cancer¹⁴, and mitochondrial myopathy¹⁵, suggesting the potential of hydrogen to prevent some age-related diseases by reducing oxidative stress. As described elsewhere^{5,8}, the increase in the intestinal production of hydrogen gas under some conditions could have beneficial effects similar or superior to those of drinking hydrogen-rich water. The possibility is also suggested that the pulsatile increase, but not continuous increase, of hydrogen concentrations could be involved in exerting its beneficial effects as a gaseous signaling modulator¹⁶. Therefore, the increased hydrogen gas concentrations found in Japanese centenarians may suggest that hydrogen gas produced in the intestine might have contributed to their longevity by preventing them from experiencing increased oxidative stress. The traditional foods and gut microbiota in Japan¹⁷⁻¹⁹, a country that is known for the longevity of its population, might be involved in such an increase of hydrogen gas production.

As shown in previous reports^{20,21}, intestinal production of hydrogen gas appeared to increase with age in the current study. It is possible that centenarians often have small-bowel bacterial overgrowth, leading to nutrient malabsorption and an increase in breath hydrogen gas²²⁻²⁴. On the contrary, considering the potential of hydrogen gas to act as a potent antioxidant in the

body, the increased production of intestinal hydrogen may have allowed centenarians to evade age-related and life-threatening diseases. Because the gut microbiota is noted in relation to aging and can be manipulated^{25,26}, the intestinal production of hydrogen gas in humans warrants further studies.

Conclusions

The breath hydrogen gas concentrations, reflecting the intestinal production, were found to be increased in Japanese centenarians. Hydrogen gas might affect longevity as an antioxidant produced by intestinal fermentation in association with the diet and the gut microbiota.

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Conflicts of Interest

The author has indicated no potential conflict of interest.

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