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Original article

Effects of ostrich meat intake on amino acid metabolism and growth hormone secretion: A comparative clinical study.

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Abstract

***Objective*:** We investigated the effects of the intake of ostrich meat (OM), which is characterized by high protein and low fat, on the body, focusing on protein and amino acid metabolism and growth hormone (GH) secretion.
***Methods*:** Plasma amino acid levels, growth hormone (GH) secretion after exercise and autonomic nervous function (device used: VM500 [Fatigue Science Laboratory Inc.]) were measured in 12 healthy subjects (33.3 ± 6.9 years old, BMI 21.6 ± 1.6) after ingestion of the test food (OM) or control food for one week each.
***Results*:** A significant increase in the blood levels of branched-chain amino acids (BCAAs), lysine and histidine, and an increase in those for 1-methylhistidine and 3-methylhistidine, which are the indicators of muscle protein breakdown, were observed after the intake of OM, suggesting that protein metabolism in the muscles was activated. Subclass analysis excluding cases with high GH before exercise load showed a significant increase in GH secretion after a walking exercise (30 min) only after the intake of OM. The autonomic nervous function test did not reveal any significant findings. There were no OM-related adverse events during the study.
***Conclusion*:** OM is a low-fat meat containing physiologically significant amino acids in large quantities, and it was suggested that OM could be safe and suitable for a protein and amino acid supplement.

***KEY WORDS*:** ostrich meat, amino acids, branched-chain amino acid (BCAA), growth hormone, anti-fatigue

Introduction

In recent years, lifestyle-related diseases such as visceral obesity, metabolic syndrome, dyslipidemia, fatty liver, and type 2 diabetes are increasing. These diseases are often accompanied by postprandial hyperglycemia (glycemic spikes), hypertriglyceridemia, and high LDL-cholesterol, resulting in a high glycative stress condition due to the increase of aldehydes derived from sugars and lipids. Glycative stress induces structural changes in the body's constituent proteins and functional proteins by highly reactive aldehyde groups, promotes the progression of arteriosclerosis, becomes a risk factor for cerebral and cardiovascular events, and is also closely related to the onset and progression of dementia.

The causes of the increase in glycative stress-associated diseases include decreased physical activity and increased caloric intake. However, qualitative changes in nutrition rather than quantitative changes in the caloric intake of Japanese people are a more serious problem. Trends of eating habits in recent years show that for carbohydrates, rice

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consumption has decreased, and for proteins, consumption of animal meat has increased rather than fish meat, suggesting that there is a slight increase in fat intake[1]. Dietary fibers have decreased with a lower intake of vegetables and fruits in the diet. As one of the methods to overcome the problems in terms of diet quality, we focused our attention on ostrich meat, which is a source of good quality protein with less fat.

An adult male ostrich (*Struthio camelus*) is 2 to meters tall and weighs over 135 kg and is the largest living bird[2-4]. Ostriches have an excellent ability to run on the ground with their kick force exerting a pressure of 4.8 tons per 100 cm^2. Ostriches are sometimes considered to be omnivorous. However, an ostrich can be defined as a herbivore based on its intestine, which is longer than other birds, where the fiber in the grass is fermented just like in horses and rabbits and used as an energy source. Stones swallowed are used as a bezoar to grind the food that has been consumed in the gizzard. Ostrich meat is rich in iron, dark red and has a crunchy texture. The meat is widely recognized as healthy meat because of its low fat content and rich L-carnitine level[5, 6]. The meat is also characterized by abundant amino acids with a sweet taste, such as alanine and glycine, compared to other meats.

A repeated measurement study was used to verify the physical changes, especially post-intake amino acid metabolism, growth hormone secretion during exercise load, and degree of fatigue, after ostrich meat (test food) ingestion with the values after control food intake as control.

Method

Subjects

The changes in growth hormone secretion and blood amino acid levels in 12 healthy men and women between 25 and 45 years old belonging or related to Urata Clinic/Sqol Kanazawa, after the intake of processed food (smoked ham, boiled in water) using ostrich meat (OM) as a test food were investigated with those after control food was taken as a control.

After the "research using human subjects" was approved by the ethics review committee of the Society for Glycative Stress Research, a briefing on the study was held at Urata Clinic/Sqol Kanazawa (Kanazawa City, Ishikawa) and 12 subjects who had given prior written consent to participate in this study and were not in conflict with the following exclusion criteria, were included as subjects:

(1) Subjects currently suffering from an illness and receiving medical treatment
(2) Subjects with a history of or now suffering from severe disease of liver, kidney, heart, lungs and blood
(3) Subjects with severe anemia
(4) Subjects who are at risk of developing an allergic reaction to the test food, or other foods or drugs
(5) Subjects who regularly consume high protein diet
(6) Female subjects who are pregnant, lactating or who might be pregnant
(7) Subjects who are currently participating in other human clinical trials, and those who have participated in other human clinical trials within the past 3 months

At the case review meeting after the completion of this study, all 12 subjects (33.3 ± 6.86 years old) who were included in this study were considered for analysis.

Study Design

The test food was smoked ham or meat boiled in water prepared using OM provided by Yoshinoya Holdings Co., Ltd. (Chuo-ku, Tokyo, Japan) (***Fig. 1***). ***Tables 1*** and ***2*** show the nutritional ingredients and amino acid content of OM.

The experiment schedule is shown in ***Fig. 2***. First, the subjects were requested to consume 100 g of the test food (OM boiled in water) as breakfast on the first day of the study. At that time, they were allowed to consume water and steamed rice along with the test food. ***Table 3*** shows the nutritional composition per serving of the test food and control food. From the time of intake until the end of the test on that day, the subjects were allowed to consume only water. Anthropometric measurements, background surveys and walking exercise with an intensity of about 3 METS were performed when the subjects visited the test facility (Visit-1). Walking conditions were the same as previously

a) **b)**

Fig. 1. **Test food (ostrich meat: OM).**
a) Smoked ham. **b)** Boiled in water.

Table 1. Nutritional composition.

Nutritional values per 100 g ostrich meat		
Energy	103	kcal
Carbohydrate	0.0	g
Ash	1.1	g
Protein	22.8	g
Fat	1.3	g
Total fatty acid	1.45	g
Saturated fatty acid	0.45	g
Monounsaturated fatty acid	0.59	g
Polyunsaturated fatty acid	0.41	g
Vitamins		
Vitamin A	0.0	μg
Vitamin B1 (thiamine)	0.18	mg
Vitamin B2 (riboflavin)	0.27	mg
Vitamin B3 (niacin)	4.38	mg
Vitamin B6	0.48	mg
Follic acid	7.00	μg
Vitamin B12	461	μg
Vitamin C	0.0	mg
Vitamin E	0.24	mg

Table 2. Amino acid composition.

Amino acid content per 100 g ostrich meat		
Arginine	1.32	g
Lysine	1.84	g
Histidine	0.54	g
Phenylalanine	0.84	g
Tyrosine	0.72	g
Leucine	1.66	g
Isoleucine	0.93	g
Methionine	0.54	g
Valine	0.96	g
Alanine	1.20	g
Glycine	0.91	g
Proline	0.79	g
Glutamic acid	3.05	g
Serine	0.84	g
Threonine	0.95	g
Aspartic acid	1.84	g
Tryptophan	0.28	g
Cystine	0.24	g
Free glutamine	0.04	g

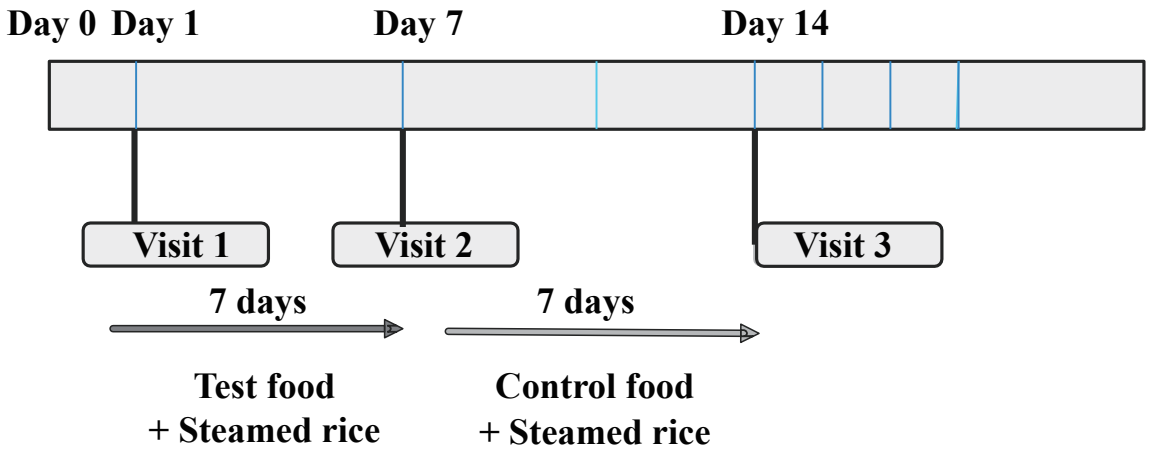


Fig. 2. **Test schedule.**

Table 3. The nutrient composition of control and test food.

	Control food	Test food	
	Cup soup	Boiled in water	Smoked ham
Water (g)	–	61.2	47.7
Energy (kcal)	136	102	96
Protein (g)	2.5	21.9	18.5
Carbohydrate (g)	22	0	0.1
Fat (g)	4.4	1.6	2.4
Sodium (mg)	659	136	323

reported [7], and the subjects walked for 30-minutes on a treadmill (Life Fitness Discover Treadmill SE3; Brunswick Corporation, Illinois, USA) set at a speed of 4.5 km/h. A glucose load (40 g of glucose) was administered 75 minutes before the walking exercise to temporarily suppress GH secretion, which is the primary endpoint. Subsequently, 15 minutes before the walking exercise, GH, amino acids analysis, triglyceride (TG), plasma glucose (PG) and immunoreactive insulin (IRI) were measured by hematology and blood biochemistry tests and autonomic nervous function was evaluated using a VM500 device (Fatigue Science Laboratory, Yodogawa-ku, Osaka, Japan). A second sample of blood was drawn after the walking exercise, and change in blood tests and autonomic nervous function before and after walking were evaluated.

The subjects, then, consumed the test food for a total of 6 days for breakfast, with 100 g intake of ham OM (test food) for 3 days, and 100 g intake of OM boiled in water (test food) for 3 days (subjects could decide the order of intake). On day 7, the subjects visited the test facility (Visit-2), a walking exercise with an intensity of about 3 METS was performed for 30 minutes. A glucose load was administered 75 minutes before the walking exercise, blood test and autonomic nervous function evaluation were performed 15 minutes before the walking exercise, and a blood test was performed again after the walking exercise.

Subsequently, the subjects consumed control food (Jikkuri Kotokoto Kongari Pan Corn Potage; Pokka Sapporo Food & Beverage Ltd., Nagoya, Aichi, Japan) that was set as the control for breakfast starting one week before the third examination day (consumption of water and steamed rice along with the control food was allowed). The tests performed on the third examination day (Visit-3) were the same as those on the second examination day. The study period was from April 2019 to May 2019.

Evaluation items

Anthropometric measurements

Height, weight, body fat percentage, body mass index (BMI), systolic and diastolic blood pressure, and pulse rate were measured. The body composition test was performed using a body composition analyzer (InBody770; InBody Japan, Koto-ku, Tokyo, Japan).

Blood chemistry examination

Peripheral blood tests and biochemical tests were performed using the blood samples. The evaluation items for the examination were GH, amino acid analysis, TG, PG and IRI. The measurements during the tests using the blood samples were performed by Alp Co., Ltd. (Kanazawa, Ishikawa, Japan).

Autonomic nervous function evaluation

Vital monitor VM500 (Fatigue Science Laboratory, Yodogawa-ku, Osaka, Japan) was used to measure the balance and amount of activity (autonomic nervous function age) of the autonomic nervous system [8,9]. In this method, sympathetic nerve (low frequency (LF)) and parasympathetic nerve (high frequency (HF)) components are extracted from the form of the pulse wave and expressed as the autonomic nerve balance (LF/HF), and an activity index of the autonomic nerves is expressed as CCVTP (coefficient of component variance total power).

The average of the calculated values for each heartbeat was used as the LF/HF. It has been reported that when a person is tired, the tension of the sympathetic nervous system increases and the activity of the parasympathetic nervous system decreases. As a result, a balance value of less than 2.0 is evaluated as "Standard value," between 2.0 and 5.0 as "Warning," and 5.0 or more as "Caution needed."

CCVTP was the average value of the indices derived from the sum of LF and HF over the entire duration of the measurement. This value is high in healthy subjects and low in those with fatigue and stress. In healthy persons, the numerical value is high while they are young and gradually decrease with aging. The result was matched with the population information of CCVTP to determine the age at which the measured value matches, and was expressed as autonomic nervous function age.

Statistical Analysis

Statistical analysis software SPSS (IBM Japan, Chuo-ku, Tokyo, Japan) was used for statistical analysis and a paired t-test was performed for comparison over time. One-way analysis of variance and Tukey's range test were performed for differences between groups. A risk rate of less than 5% was considered a significant difference. No outliers in particular were set. However, when data could not be obtained due to problems during testing or a serious problem occurred in the reliability of the data, such values were considered as missing values and substitute values were not used.

Ethical Review

This study was conducted in compliance with the Helsinki Declaration (revised at the 2013 WMA General Assembly in Fortaleza) and the Ethical Guidelines for Medical and Health Research Involving Human Subjects (Notification of the Ministry of Education, Culture, Sports, Science and Technology Ministry of Health, Labor and Welfare). This study was approved by holding a "ethics committee meeting for 'Research on Human Subjects' at the Society for Glycative Stress Research" (Shinjuku-ku, Tokyo, Japan), where deliberation on the ethics and validity of the study was conducted. (Glycative Stress Research 2019 No. 001). The clinical trial for this study was pre-registered (UMIN #000035656).

Results

General background

The 12 subjects analyzed were 33.3 ± 6.9 years old, 169.7 ± 7.6 cm tall, weighing 62.6 ± 8.5 kg, and having a BMI of 21.6 ± 1.6 (***Table 4***).

Table 4. Anthropometry.

	Average	SD
Age	33.3 ±	6.86
Height cm	169.7 ±	7.59
Weight kg	62.6 ±	8.51
Body fat %	21.5 ±	5.57
BMI –	21.6 ±	1.55

n = 12. BMI, body mass index; SD, standard deviation.

Growth Hormone (GH)

GH on Visit-1 (day 1 of OM intake) was 0.86 ± 1.09 ng/mL and 3.20 ± 3.69 ng/mL before and after the walking exercise. On Visit-2 (day 7 of OM intake), it was 1.23 ± 1.66 ng/mL and 2.45 ± 2.01 ng/mL before and after the walking exercise On Visit-3 (day 7 of control intake), it was 1.13 ± 2.46 ng/mL and 1.71 ± 1.80 ng/mL before and after the walking exercise (***Table 5***). The changes in blood GH levels between before and after exercise on each visit are shown in ***Fig. 3***. There was no significant difference in the GH change rate between the groups in Visit-1, Visit-2 and Visit-3.

During the verification of individual data for each visit, some subjects were found to have a high GH value before exercise. Hence, subclass analysis was performed with the pre-exercise cutoff value set at 4.00 ng/mL or more (***Fig. 4***). During Visit-2, GH had increased significantly after exercise ($p = 0.018$, ***Table 6***). On the other hand, no significant increase in GH was observed during Visit-1 and Visit-3.

Amino acid analysis

In the blood amino acid analysis performed during each visit (***Table 7***), amino acid that showed significant difference between groups on Visit-2 and Visit-3 were valine ($p < 0.05$), methionine ($p < 0.05$), isoleucine ($p < 0.01$), leucine ($p < 0.01$), tyrosine ($p < 0.05$), β-alanine ($p < 0.05$), 1-methylhistidine ($p < 0.01$), lysine ($p < 0.05$), and 3-methylhistidine ($p < 0.01$), and all the values during Visit-2 were significantly higher than those during Visit-3. 1-methylhistidine and 3-methylhistidine were significantly higher during Visit-1 than during Visit-3 (both $p < 0.01$, ***Fig. 5***). These amino acids remained high during ostrich meat intake, but showed a significant decrease one week after discontinuation.

Triglyceride (TG)

Serum TG was 88.3 ± 34.9 mg/dL before exercise and 71.2 ± 25.4 mg/dL after exercise during Visit-1, 100.2 ± 77.2 mg/dL before exercise and 65.2 ± 18.3 mg/dL after exercise during Visit-2, and 78.1 ± 26.5 mg/dL before exercise and 74.3 ± 36.6 mg/dL after exercise during Visit-3 (***Table 8***). There was no significant difference in the TG change rate between the groups during Visit-1, Visit-2, and Visit-3.

Blood glucose (PG)

PG was 114.0 ± 35.5 mg/dL before exercise and 93.1 ± 15.9 mg/dL after exercise during Visit-1, 112.2 ± 35.3 mg/dL before exercise and 88.8 ± 14.8 mg/dL after exercise during Visit-2, and 108.3 ± 26.7 mg/dL before exercise and 92.8 ± 18.3 mg/dL after exercise during Visit-3 (***Table 8***). There was no significant difference in the PG change rate between the groups during Visit-1, Visit-2, and Visit-3.

Table 5. Serum GH

		Before walking	After walking	p value	Inter-group analysis (vs.Visit-3)
Visit-1 (OM 1 day)	ng/mL	0.86 ± 1.09	3.20 ± 3.69	0.056	0.481
Visit-2 (OM 7 days)	ng/mL	1.23 ± 1.66	2.45 ± 2.01	0.134	0.382
Visit-3 (Control 7 days)	ng/mL	1.13 ± 2.46	1.71 ± 1.80	0.538	

Data are expressed as mean ± SD, paired t test, n = 12. OM, ostrich meat; Control, control meat; GH, growth hormone; SD, standard deviation. Statistical analysis by Turkey test.

Table 6. Serum GH: Subclass analysis.

		Before walking	After walking	p value
Visit-1 (OM 1 day)	ng/mL	0.86 ± 1.09	3.20 ± 3.69	0.056
Visit-2 (OM 7 days)	ng/mL	0.81 ± 0.95	2.62 ± 2.02	**0.018**
Visit-3 (Control 7 days)	ng/mL	0.40 ± 0.46	1.39 ± 1.53	0.064

Subclass analysis was conducted by excluding the subjects who' s GH values exceeded 4.00 ng/mL before walking.
Data are expressed as mean ± SD, paired t test, Visit-1 (n = 12), Visit-2, 3 (n = 11).
OM, ostrich meat; Control, control meat; GH, growth hormone; SD, standard deviation.

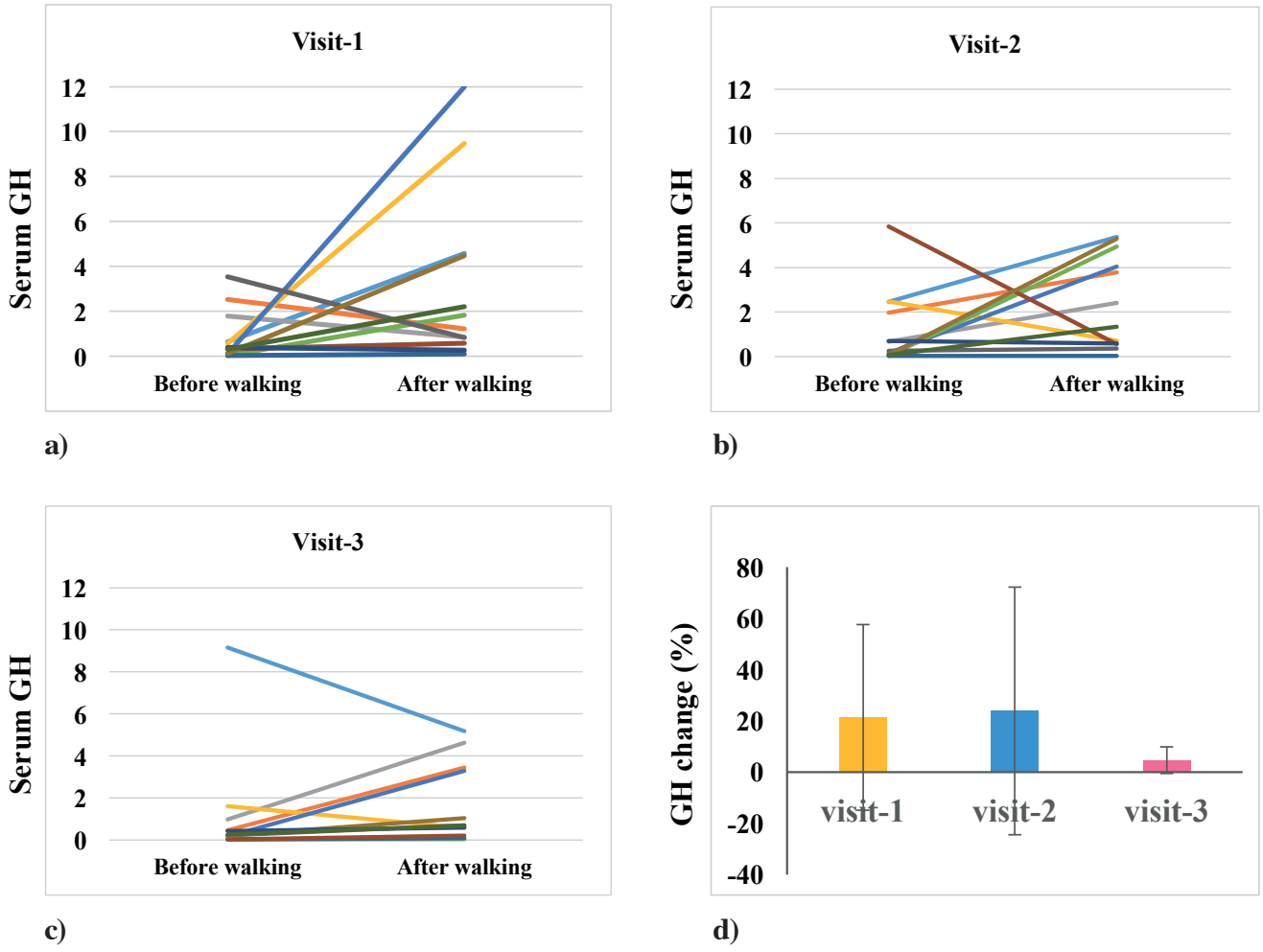


Fig. 3. **Exercise-induced GH secretion: Total analysis.**

a) One day after Test food (OM) intake (Visit-1). **b)** One week after Test food (OM) intake (Visit-2). **c)** One week after Control food intake (Visit-3). **d)** Percent change of serum GH. Results are expressed as mean ± SD. n = 12 at each visit. GH, growth hormone; OM, ostrich meat; SD, standard deviation.

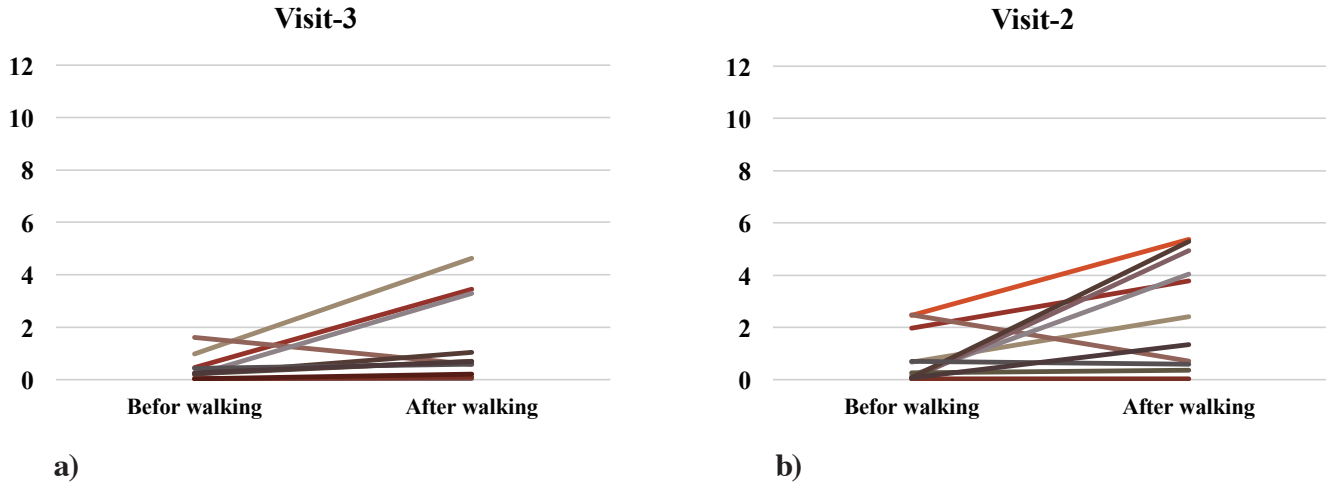


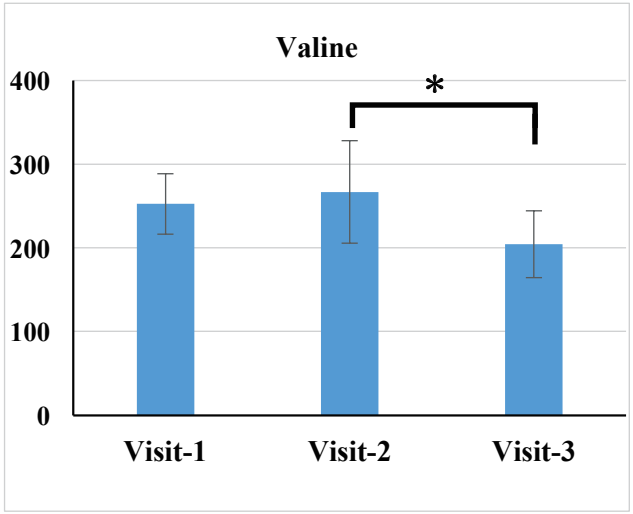
Fig. 4. **Exercise-induced GH secretion: Subclass analysis.**

a) One week after Test food (OM) intake (Visit-2). **b)** One week after Control food intake (Visit-3). The subjects with high pre-values of GH exceeding 4.00 ng/mL are excluded in the subclass analysis. *$p < 0.05$, paired t test, n = 11. GH, growth hormone; OM, ostrich meat.

Table 7. Amino acid Analysis

	Visit-1 (OM 1 day)	Visit-2 (OM 7 days)	Visit-3 (Control 7 days)
Taurine	77.23 ± 9.82	76.82 ± 9.08	73.31 ± 13.83
Phosphoethanolamine	– ± –	– ± –	– ± –
Aspartic acid	4.46 ± 0.84	4.33 ± 1.28	4.14 ± 0.79
Hydroxyproline	13.48 ± 3.15	13.56 ± 4.45	10.75 ± 5.52
Threonine	157.83 ± 27.51	156.38 ± 45.00	135.09 ± 24.83
Serine	132.88 ± 18.83	132.38 ± 19.53	124.66 ± 11.69
Asparagine	65.83 ± 11.59	68.01 ± 11.60	62.83 ± 10.09
Glutamic acid	61.20 ± 11.93	60.93 ± 17.59	62.93 ± 17.20
Glutamine	512.01 ± 66.17	521.92 ± 69.32	505.34 ± 43.99
Sarcosine	– ± –	– ± –	– ± –
α-Aminoadipic acid	– ± –	– ± –	– ± –
Proline	178.21 ± 44.81	180.82 ± 50.56	168.31 ± 55.11
Glycine	241.76 ± 37.76	230.84 ± 41.97	234.48 ± 37.25
Alanine	433.88 ± 75.46	434.88 ± 80.11	414.98 ± 47.72
Citrulline	23.26 ± 2.69	24.66 ± 4.85	21.74 ± 3.56
α-Aminobutyric acid	23.59 ± 6.15	24.98 ± 5.99	22.59 ± 4.92
Valine	252.58 ± 36.09	266.95 ± 61.21	204.49 ± 39.90
Cystine	30.42 ± 5.19	31.23 ± 6.28	32.78 ± 5.73
Methionine	28.14 ± 6.78	30.25 ± 9.66	20.54 ± 4.60
Cystathionine	– ± –	– ± –	– ± –
Isoleucine	71.00 ± 16.68	81.05 ± 32.26	49.08 ± 9.88
Leucine	128.13 ± 26.07	144.36 ± 46.11	98.01 ± 17.86
Tyrosine	66.73 ± 13.98	69.56 ± 12.94	54.69 ± 14.61
β-Alanine	9.79 ± 1.88	10.38 ± 3.43	7.42 ± 1.84
Phenylalanine	65.20 ± 12.57	66.33 ± 8.89	57.58 ± 12.07
β-Aminoisobutyric acid	2.04 ± 0.81	1.89 ± 0.71	2.08 ± 0.79
Homocystin	– ± –	– ± –	– ± –
γ-Aminobutyric acid	– ± –	– ± –	– ± –
Monoethanolamine	9.89 ± 1.83	9.27 ± 1.06	8.67 ± 1.43
Hydroxylysine	– ± –	– ± –	– ± –
Ornithine	129.04 ± 25.45	133.37 ± 39.60	108.90 ± 26.23
1-Methyl histidine	18.11 ± 4.61	22.93 ± 9.52	7.50 ± 4.98
Histidine	92.29 ± 13.79	92.74 ± 13.83	84.95 ± 13.74
Lysine	223.84 ± 55.00	236.96 ± 73.64	178.20 ± 25.78
3-Methyl histidine	6.20 ± 1.26	6.51 ± 1.42	4.37 ± 1.21
Tryptophan	56.07 ± 9.89	56.17 ± 8.88	48.31 ± 9.32
Anserine	– ± –	– ± –	– ± –
Carnosine	– ± –	– ± –	– ± –
Arginine	41.30 ± 15.42	45.28 ± 13.03	33.28 ± 13.86
Alloisoleucine	– ± –	– ± –	– ± –
Fischer' s ratio	3.51 ± 0.74	3.59 ± 0.63	3.23 ± 0.70

n = 12. OM, ostrich meat; Control, control meat; Fischer' s ratio, the molar ratio of BCAAs (leucine, valine, isoleucine) to aromatic AAs (phenylalanine, tyrosine); BCAAs, branched-chain amino acids; SD, standard deviation.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Visit-1
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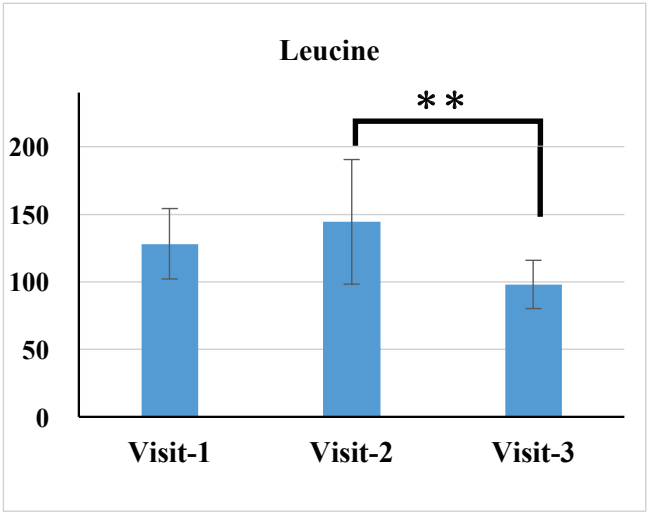
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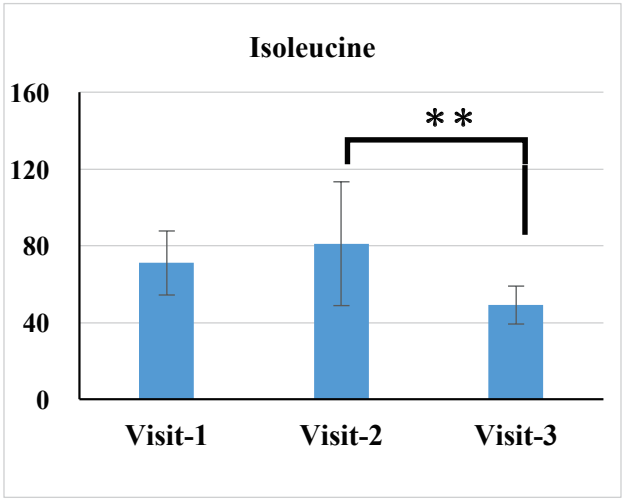
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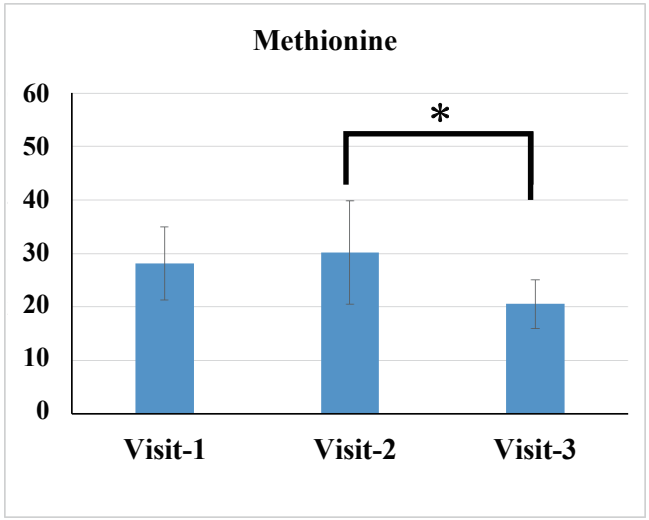
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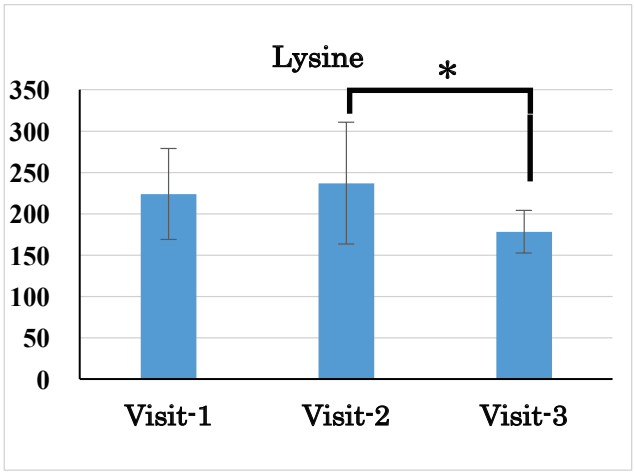
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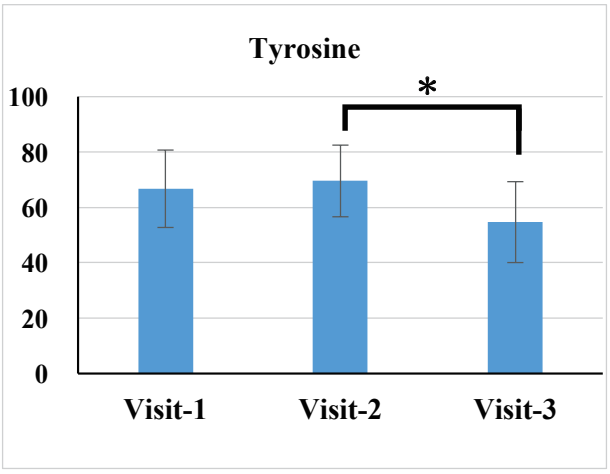
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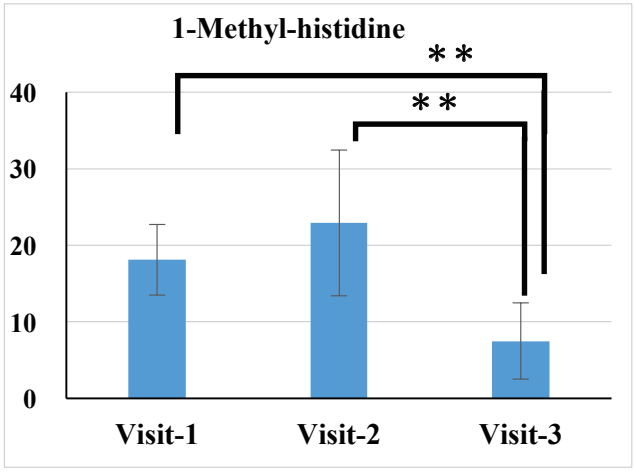
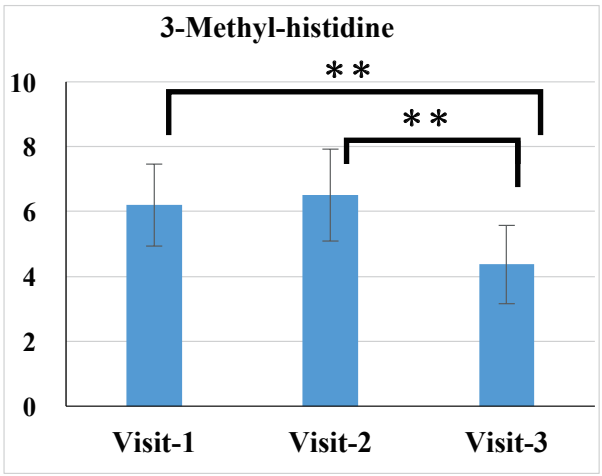


g)



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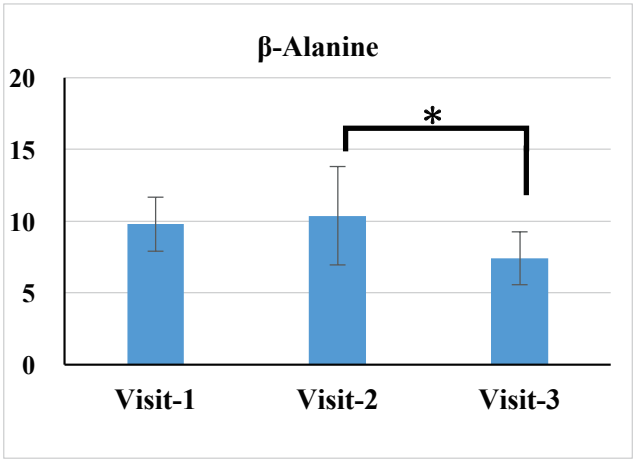

i)

Fig. 5. **Changes of plasma amino acid.**

a) Valine. **b)** Leucine. **c)** Isoleucine. **d)** Methionine. **e)** Lysine **f)** Tyrosine. **g)** 1-Methyl-histidine. **h)** 3-Methyl-histidine. **i)** β-Alanine. Results are expressed as mean ± SEM, n = 12. *p < 0.05, **p < 0.01 by Tukey's test. SEM, standard error mean.

Insulin (IRI)

Serum IRI was 26.4 ± 10.9 μU/mL before exercise and 11.0 ± 8.0 μU/mL after exercise during Visit-1, 23.5 ± 10.0 μU/mL before exercise and 10.7 ± 6.6 μU/mL after exercise during Visit-2, and 22.2 ± 9.2 μU/mL before exercise and 10.8 ± 6.8 μU/mL after exercise during Visit-3. In each group, insulin decreased significantly before and after exercise (both $p < 0.01$, ***Table 8***). There was no significant difference in the IRI change rate between the groups during Visit-1, Visit-2, and Visit-3.

Autonomic nervous function evaluation

Autonomic nerve balance (LF/HF) was 1.70 ± 1.71 before exercise and 2.57 ± 2.11 after exercise during Visit-1, 1.01 ± 0.63 before exercise and 2.00 ± 1.41 after exercise during Visit-2, and 1.46 ± 1.17 before exercise and 1.46 ± 0.82 after exercise during Visit-3. During Visit-2, LF/HF increased significantly before and after exercise ($p = 0.045$, ***Table 9***). However, those values were mostly around the "Standard value (less than 2.0)." There was no significant difference in the LF/HF change rate between the groups during Visit-1, Visit-2, and Visit-3.

Among the pre-exercise LF/HF values of Visit-1, Visit-2, and Visit-3, the values during Visit-2 were the lowest. However, no significant difference was observed between the groups.

The autonomic nervous function age was 35.6 ± 14.0 years before exercise and 40.3 ± 13.4 years after exercise during Visit-1, 44.4 ± 11.2 years before exercise and 42.5 ± 9.2 years after exercise during Visit-2, and 41.7 ± 11.4 years before exercise and 38.2 ± 12.1 years after exercise during Visit-3. There was no significant difference in the change rate between the groups during Visit-1, Visit-2, and Visit-3.

Table 8. Serum TG, PG and Serum IRI

			Before walking	After walking	p value
serum TG	Visit-1 (OM 1 day)	mg/dL	88.25 ± 34.88	71.17 ± 25.36	0.202
	Visit-2 (OM 7 days)	mg/dL	100.17 ± 77.23	65.17 ± 18.26	0.158
	Visit-3 (Control 7 days)	mg/dL	78.08 ± 26.52	74.25 ± 36.56	0.781
PG	Visit-1 (OM 1 day)	mg/dL	114.00 ± 35.52	93.08 ± 15.85	0.088
	Visit-2 (OM 7 days)	mg/dL	112.17 ± 35.30	88.75 ± 14.84	0.055
	Visit-3 (Control 7 days)	mg/dL	108.25 ± 26.72	92.83 ± 18.30	0.129
Serum IRI	Visit-1 (OM 1 day)	μU/mL	26.37 ± 10.94	11.03 ± 7.95	**0.001**
	Visit-2 (OM 7 days)	μU/mL	23.49 ± 10.01	10.65 ± 6.57	**0.002**
	Visit-3 (Control 7 days)	μU/mL	22.24 ± 9.17	10.83 ± 6.82	**0.003**

Data are expressed as mean ± SD, paired t test, n = 12. OM, ostrich meat; Control, control meat; TG, triglyceride; PG, plasma glucose; IRI, immunoreactive insulin; SD, standard deviation.

Table 9. Evaluation of autonomic nervous activity.

			Before walking	After walking	p value
Autonomic balance	Visit-1 (OM 1 day)		1.70 ± 1.71	2.57 ± 2.11	0.296
	Visit-2 (OM 7 days)		1.01 ± 0.63	2.00 ± 1.41	**0.045**
	Visit-3 (Control 7 days)		1.46 ± 1.17	1.46 ± 0.82	0.997
Autonomic nervous function age	Visit-1 (OM 1 day)	year	35.58 ± 13.98	40.25 ± 13.39	0.433
	Visit-2 (OM 7 days)	year	44.42 ± 11.22	42.50 ± 9.24	0.666
	Visit-3 (Control 7 days)	year	41.67 ± 11.40	38.17 ± 12.08	0.492

Data are expressed as mean ± SD, paired t test, n = 12. OM, ostrich meat; Control, control meat; GH, growth hormone; SD, standard deviation.

Safety evaluation

No adverse events or side effects were observed due to the test food during or after the study period.

Discussion

How ostrich meat became a food source

The murals of ancient Egypt depict ostriches being raised. After landing in Cape Town, South Africa in 1652, the Dutch actively captured and slaughtered ostriches, like other wild birds. Ostrich breeding became active around the 17th century and by the 20th century, ostrich feathers, along with gold, diamonds and wool, had become South Africa's principal item of trade. Ostrich breeding had long been a livestock business that was monopolized by South Africa. However, the ban on the export of eggs and birds from South Africa was lifted in 1993, and breeding of ostriches as new poultry source became widespread throughout the world. Even in Japan, since the late 1990s, ostrich breeding has become more active with an increase in the number of birds and establishment of producers' groups, and the ostrich was included as an animal subject in the Livestock Infectious Disease Prevention Act in 2008 [10].

Since ostrich meat (OM) is high in protein and low in fat, some consumers who are conscious about health started consuming OM as a substitute for beef in Europe and the United States, especially in the European Union (EU) countries, Bovine Spongiform Encephalopathy (BSE) was a tailwind for OM. The market size based on consumption is estimated to be tens of thousands of tons per year worldwide and about 100 tons per year in Japan.

Result summary

In this study, blood amino acids, GH secretion and degree of fatigue (autonomic nervous function) after exercise load were compared by repeated measurements in 12 healthy men and women after intake of OM (test food) and control food. The results showed that blood levels of amino acids such as branched-chain amino acids (BCAAs) increased and GH secretion was observed to increase significantly

after exercise load with subclass analysis when OM was consumed. The autonomic nervous function test showed no significant difference between the groups. No adverse events were observed with OM intake.

Branched-chain amino acids (BCAAs)

BCAAs, namely, such as valine, leucine and isoleucine, are amino acids involved in muscle synthesis, and the blood levels of which increased after OM intake in this study. Post-exercise intake of BCAA activates muscle protein synthesis-associated signaling in human skeletal muscles and suppresses exercise-induced activation of mechanisms associated with proteolysis[11]. The administration of BCAAs suppresses the exercise-induced expression of the mTORC1 inhibitor DDIT4 mRNA in skeletal muscles and prevents activation of the ubiquitin-proteasome system. Post-exercise consumption of BCAAs partially suppresses exercise-induced expression of peroxisome proliferator-activated receptor γ-coactivator 1α mRNA and activation of ubiquitin-proteasome signaling and suppresses DDIT4 mRNA expression. On the other hand, ACC Ser79/222 phosphorylation, an endogenous marker of AMP-activated protein kinase activity, increases after exercise regardless of BCAA intake. Key markers of protein synthesis, such as expression of IGF1 mRNA isoforms or phosphorylation do not change with or without BCAA intake.

Maintaining skeletal muscles is important for sustaining daily physical activity and maintaining quality of life. Protein increases muscle protein synthesis within 1-2 hours after intake. Muscle protein anabolism associated with protein intake is caused mainly by leucine, and the rate of muscle protein synthesis also increases with an increase in the blood leucine levels[12]. Intake of leucine-enriched essential amino acid mixture suppresses the exercise-induced elevation of muscle damage markers in the blood, reduces muscle damage and assists muscle recovery[13].

When electrical stimulation of skeletal muscles and BCAA intake are used in combination for healthy students, lactic acid levels after exercise are lower, oxygen consumption is higher, and delayed myalgia is milder compared to the control group[14]. In the elderly with sarcopenia who are undergoing convalescent rehabilitation, a combination of resistance training and BCAA intake brings better improvement[15]. There are several other reports on the recovery-promoting effects of the intake of BCAAs on patients in rehabilitation[16,17]. Since BCAA levels also decrease after invasive surgeries[18], OM may be suitable as a preoperative and postoperative meal for BCAA supplementation.

Association with GH secretion

Since basic amino acids such as lysine and arginine have a GH secretion effect, intravenous administration tests of these amino acids are performed to diagnose GH secretion deficiency[19]. These amino acids also affect the gastrointestinal motility, while lysine promotes gastrointestinal motility, but arginine suppresses gastrointestinal motility.

In clinical studies, it has been reported that a single intake of a supplement containing lysine and arginine also increases GH secretion after exercise load (30-minute ergometer)[20]. In this study, there was a significant increase in lysine blood levels as well as GH secretion during subclass analysis after OM intake, suggesting that the potential of GH secretion may have increased after OM intake.

Muscle metabolism and 3-methylhistidine

In the body, proteins maintain a nitrogen balance state by repeating synthesis and decomposition. Skeletal muscle tissues account for a large percentage of body proteins and have a high metabolic rate. 3-Methylhistidine is a constituent amino acid of myosin and actin. 3-methylhistidine produced by the decomposition of muscle protein is excreted in urine without being metabolized[21]. By measuring the amount of 3-methylhistidine excreted in the urine, the decomposition rate of actin in the muscles can be estimated. The synthesis of 3-methylhistidine in muscle cells is performed by introducing the methyl group of methionine into histidine after the translation of the polypeptide chain of myosin and actin[22,23].

As protein intake in the diet increases, fluctuations in enzyme activity increase[24,25], plasma protein and proteins in the liver, kidney, and small intestinal mucosa[25] increase, and protein metabolism in the muscles increase[21]. It is considered that the rate of proteolysis of skeletal muscles increases as muscle metabolic activity increases[27]. In this study, since amino acids, such as BCAAs, which assist muscle synthesis, increased with the intake of OM, indicating that protein metabolism in the skeletal muscles was activated, it can be interpreted that 3-methylhistidine, which is an index of muscle protein decomposition, increased.

Fatigue and amino acids

A close relationship exists between amino acids and fatigue. BCAA concentration in the blood decreases during acute muscle fatigue due to exercise load[28]. Valine supplementation during exercise is effective in maintaining hepatic glycogen and blood glucose, increases spontaneous activity after exercise, and contributes to reducing fatigue in animal experiments using rats[29]. It has been reported in a randomized, double-blind, placebo-controlled, crossover study that a 4-week intake of food containing S-allyl cysteine reduces fatigue[30]. Even clinical studies have shown that intake of leucine-enriched essential amino acid mixture assists in the recovery from muscle fatigue[13]. The amino acid composition of OM is rich in leucine and may contribute to recovery from muscle fatigue.

In this study, a reduction in fatigue could be expected by OM intake. However, a significant difference was not detected between the groups in autonomic nervous function evaluation. The autonomic nerve balance (LF/HF values) before exercise load was 1.70 ± 1.71 during Visit-1, 1.01 ± 0.63 during Visit-2, and 1.46 ± 1.17 during Visit-3, and the post-OM intake values were the lowest. This could be an indicator of a potential fatigue-reducing effect of OM intake.

Safety

The investigation of radioactive contamination following the accident at the Fukushima Daiichi Nuclear Power Station has been reported[31]. As a result of inspection using ostrich samples collected from December 2011 to May 2012, ^{129m}Te and ^{110m}Ag were not detected in any of the inspected samples. Low levels of ^{137}Cs (19.2 ± 11.7 Bq/kg) and ^{137}Cs

(18.3 ± 11.3 Bq/kg) were detected in the skeletal muscles of five ostriches bred in the Kanto region, 150 km away from the nuclear power plant while no radioactive Cs was detected in the ostriches bred before the accident, and it is presumed that the cause of the contamination was a transient event caused by the radioactive material carried from the nuclear power plant. Radiation contamination has not been reported in ostriches hatched after 2013. Since ostrich meat is rich in high quality unsaturated fatty acids, which are oxidized due to long-term storage, the meat needs to be stored carefully [4].

Conclusion

The result of comparative study of blood amino acids, GH secretion after exercise load and autonomic nervous function after intake of test food (OM) and control food for one week each on 12 healthy subjects showed a significant increase in blood amino acid levels, such as BCAAs, lysine and histidine, and a significant increase in GH secretion after exercise load with subclass analysis, after OM intake. OM is low-fat animal meat containing good quality amino acids in large quantities, and it was suggested that OM is safe and suitable as a protein and amino acid supplement.

Conflict of Interest

This research received support from Yoshinoya Holdings.

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