

HEALTH AND NUTRITION NEWS

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Table of Contents

CURRENT RESEARCH PROJECTS

Focusing On What We Call Caspian Sea Yogurt..... **Tatsuya Ishida**

THOUGHTS ON HEALTH AND NUTRITION RESEARCH

Can Intake Of Antioxidants Reduce Immune System Ageing?..... **Naomi Aiba**

RESEARCH FINDINGS

Skeletal muscle FOXO1 (FKHR)-transgenic mice have less skeletal muscle mass, down-regulated type I (slow twitch / red muscle) fiber genes, and impaired glycemic control..... **Yasutomi Kamei**

Effective Health and Nutrition Materials towards Occupation Group..... **Katsushi Yoshita**

Gene Mest Which Enlarges Adipose Cell..... **Osamu Ezaki**

Women with high BMI tend to underreport energy intake –Study for new female university students aged 18-20 years–..... **Hitomi Okubo**

Increasing Thinness among Young Women..... **Hidemi Takimoto**

Current Research Projects

Focusing On What We Call Caspian Sea Yogurt

Tatsuya Ishida
Division of Applied Food Research

In the Division of Applied Food Research where I belong to, surveys examinations and tests of nutrient and other composition, regarding registration and approval of Food for Special Dietary Uses such as foods for the ill sick diet or food products for specified health use, are conducted. And researches of effects influences of nutrients and other food components of food to on the human body are on going in large part. I am in charge of all the activities regarding organisms such as measurement of vitamin B6, vitamin B12, and niacin using bacteria, or counting of bacteria such as bifidobacteria or lactic acid bacteria in yogurt of food for specified health use or lactic acid bacteria beverage. And we also conduct research on what we call Caspian Sea Yogurt as related activities.

Some of you may know that Caspian Sea Yogurt is the yogurt that Dr. Yukio Iemori, professor of emeritus at Kyoto University brought back from Caucasia. It has feature of less acidity and sticky which differs from other yogurt on the market. Also, it is accepted in each family and people have a broad interest in handing on by repackaging into smaller because it is easy to produce by transferring some portion to milk. Then I looked up the feature of the bacteria.

First of all, I tried identification of the including bacteria by testing DNA sequences and homology of base sequence and DNA. Two kinds of bacteria, *Lactococcus lactis* subsp. *cremoris* and *Acetobacter orientalis* were found. Generally, bifidobacteria *Bifidobacterium bifidum*, lactobacilli *Lactobacillus*, and *Streptococcus thermophilus* are mainly included in yogurt and lactic acid bacteria beverage. Bacteria of Caspian Sea Yogurt differs differ from any of those. Furthermore, past examples of reports on source and ground of separation isolation about these bacteria indicate that *Lactococcus lactis* subsp. *cremoris* and *Acetobacter orientalis* are were mainly separated isolated from starter of cheese and soybean-fermented food, respectively. Both were founded as bacteria which were separated isolated from food. However, *Acetobacter orientalis* have been separated isolated only in Indonesia up till now,

and the idea that there is no example of separation isolation from daily products generates interests to our future researches. And *Lactococcus lactis* subsp. *cremoris* are antibacterial had antimicrobial activity against to some of other lactic acid bacteria.

In the first place, I got attracted to this yogurt by accident. I have come to work for this institute since two years ago, and conducted the research on probiotex probiotics including lactic acid bacteria bacterium. In the meantime, I have mainly done the research on evolution and system phylogeny of cyanobacteria (blue-green bacterium bacteria: the first organisms on earth which carried out oxygen generating photosynthesis), and I also have studied on common bacteria germ, not taking particular note of lactic acid bacterium bacteria. When I had a hard time deciding subject of my study on that account, prior co-workers introduced me Caspian Sea Yogurt, saying "try some interesting yogurt that I eat at home". When I tried it, it was had viscosity thick, no acidity, and aroma of yeast. I haven't seen any yogurt like it. But I just felt this was an interesting fermented daily milk product, and kept adding milk on it so that I never thought of as reporting like this.

By the way, aroma of Caspian Sea Yogurt that I've got from prior co-worker seemed a little differ from one I am researching now since origin were different so that other microorganisms germ may existed. Although I really want to find them out, I didn't realize to lost it, I'm ashamed to say. It's really too bad that it is difficult to take a look, but I want to check it out when I get rare fermented daily product some day.



Thoughts on Health and Nutrition Research

Can Intake Of Antioxidants Reduce Immune System Ageing?

Naomi Aiba
Division of Applied Nutrition

According to abridged life table for 2003, reported in July this year, Japan is the country where people have the longest life expectancy in the world in which current life expectancy for men and women are 78.6 and 85.33 years old respectively. When we look at the life expectancy table all over the world, women live longer than men in each country. Why is there gender difference in life expectancy around the world? Since causes of death are based on various factors such as biological, sociological, environmental and so forth, it seems impossible to state just the single reason for this phenomenon. But immune system is necessary for people's health maintenance, and lowering the mortality rate by infectious diseases greatly involves in prolonged life expectancy, so we are going to take a look at this gender difference of life expectancy from viewpoint of immune system.

Immunity starts from distinguishing between self and not-self, and it excretes not-self. In this case, for example, not-self is the source of infection (virus or bacteria) or infected cell in infectious disease, and cancer originated in the body is regarded as same. If not-self can't be recognized properly, the immune system doesn't work properly. As a result, various diseases are developed, and cure will be difficult. Here is a reason why the immune function is cited as a big factor which influences on the fate of organisms. The immune function is mainly found in cell-mediated immunity, especially in the T lymphocyte functions. T lymphocytes differentiate and proliferate within the thymus, and move to peripheral blood and lymph node. The thymus, in which the T lymphocytes differentiate and mature, gets bigger during

growth period. After the maturation, age-associated thymic atrophy occurs and results in the inefficient functioning of the immune system. In this regard, there is a gender difference on a period when thymic atrophy occurs, and thymic atrophy with male apparently occurs in earlier age, suggesting that the decline of immune function in male occur earlier than that in female. Thymic atrophy can be treated as one of the signs of aging, and immune system ageing in male occurs earlier than that in female. Intracellular reactive oxygen species are thought as candidates for ageing causes of cells including thymocyte. Both elimination of reactive oxygen species and antioxidant capacity towards reactive oxygen species are known to influence on aging and falling lifestyle related diseases. Both the activities of antioxidant enzymes and antioxidant components have antioxidant ability within an organism, and both can be controlled by various nutrition components in foods. Antioxidant enzymes need metals such as selenium, zinc, and magnesium for their activities and also the intake of antioxidant-related nutrients can enhance antioxidant state within the cell. Therefore, antioxidant components in the food are focused as an anti-ageing factor. Recently, the state of intracellular antioxidant is reported to influence to immune balance and antioxidant components are expected to be a factors to regulate immune function. Proper intake of food containing antioxidants may control thymic atrophy and maintain immune system, and the early decline of immune function with male may be delayed. Eventually, it may realize that men have the same level of life expectancy as women.

Table Life Expectancy by countries and genders

country	men	women	difference	country	men	women	difference
Japan	78.36	85.33	6.97	Australia	75.59	81.58	5.99
Hong Kong	78.60	84.50	5.90	Germany	75.38	81.22	5.84
Switzerland	77.40	83.00	5.60	England	75.68	80.39	4.71
France	75.20	82.80	7.60	South Korea	72.84	80.01	7.17
Italy	76.54	82.51	5.97	USA	74.40	79.80	5.40
Iceland	78.70	82.50	3.80	China	69.93	73.33	3.40
Canada	77.00	82.10	5.10	Russia	59.00	72.30	13.30
Sweden	77.30	82.00	4.70	India	60.40	61.80	1.40
Norway	77.04	81.93	4.89				

Research Findings

Skeletal muscle FOXO1 (FKHR)-transgenic mice have less skeletal muscle mass, down-regulated type I (slow twitch / red muscle) fiber genes, and impaired glycemic control.

Yasutomi Kamei
Division of Clinical Nutrition

Skeletal muscle is the largest organ in the human body, comprising about 40% of body weight. The mass and composition of skeletal muscle is critical for its functions, such as exercise, energy expenditure and glucose metabolism. Skeletal muscle mass and composition are plastic and affected by physical activity, environmental factors, or pathological conditions; for example, skeletal muscle mass is reduced by lack of muscle use in a long-term bedridden condition. This skeletal muscle loss is called atrophy. Atrophy is also observed during starvation, cachexia, severe diabetes and aging. However, the precise mechanism of atrophy is unclear.

FOXO1, a member of the FOXO forkhead type transcription factors and co-factor of nuclear hormone receptor, is markedly up-regulated in skeletal muscle in energy-deprived states such as fasting and severe diabetes, but its functions in skeletal muscle remain poorly understood. In this study, we created transgenic mice specifically overexpressing FOXO1 in skeletal muscle. These mice weighed less than the wild-type control mice, had a reduced skeletal muscle mass, and the muscle was paler in color (Fig. 1). Microarray analysis revealed that the expression of many genes related to the structural proteins of type I muscles (slow twitch, red muscle) were decreased. Histological analyses showed a marked decrease in size of both type I and type II fibers, and a significant decrease in the number of

type I fibers in the skeletal muscle of FOXO1 mice. Enhanced gene expression of a lysosomal proteinase, cathepsin L, which is known to be up-regulated during skeletal muscle atrophy, suggested increased protein degradation in the skeletal muscle of FOXO1 mice. Running wheel activity (spontaneous locomotive activity) was significantly reduced in FOXO1 mice compared to control mice. Moreover, the FOXO1 mice showed impaired glycemic control after oral glucose and intraperitoneal insulin administration.

Then, we examined the change in endogenous FOXO1 expression caused by physical inactivity. The right hindlimbs of wild type mice were immobilized in plaster casts, and the left hindlimbs were left freely moving as a control. After three weeks in the plaster casts, the skeletal muscle weight of the right hindlimbs was significantly lighter than that in the controls. The expression of type I genes, but not type II genes, was markedly decreased in the plaster-casted muscle. At the same time, endogenous FOXO1 mRNA was increased in the immobilized muscle. Namely, the change in gene expression observed in the plaster-casted skeletal muscle was similar to that observed in the FOXO1 mice. These results suggest that FOXO1 negatively regulates skeletal muscle mass and type I fiber gene expression, and leads to impaired skeletal muscle function. Activation of FOXO1 appears to be involved in the pathogenesis of muscle atrophy.

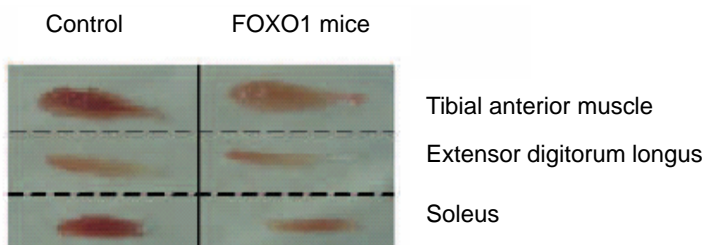


Figure1. Photo of "FOXO1 mice" muscle.

Soleus and tibial anterior muscle of control mice are red (left), while those of FOXO1 mice are paler (right). Extensor digitorum longus muscle is composed of type II fibers, less change was observed between control and FOXO1 mice.

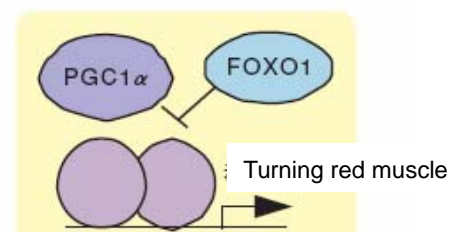


Figure2. A schematic model.

PGC-1 α causes type I fiber formation. As the FOXO1 protein can interact with the PGC-1 α protein, FOXO1 may affect certain functions of PGC-1 α . A certain nuclear receptor(s) and transcription factor(s), which can interact with both FOXO1 and PGC-1 α , may be involved in a process positively and negatively regulated by PGC-1 α and FOXO1.

Research Findings

Effective Health and Nutrition Materials towards Occupation Group

Katsushi Yoshita
Division of Health and Nutrition Monitoring

Appropriate nutrition intake can not be lacked to maintenance and promotion of good health, and prevention of disease. Thus, it is necessary for subjects of individuals and groups to learn and practice preferable diet life and lifestyle. And it is necessary to convey the right information. But kinds of materials to gain more attention and effective are hardly exposed. So we provided 3 kinds of information of health and nutrition materials, and attract attentions.

Methods of information service are following 3 materials:

- 1) Point of purchase advertising menus (POP menus)
A5 size, horizontal, colored, written on the menu holder.
Change contents every week, set on every table of staff's dining room.
Basic configuration of space is title or promo word at upper 15% part, comments at 45% of page, and related illustrations at rest of 40%.
- 2) Posters
A3 size, vertical, colored.
Change contents every 4-8 weeks.
Show at appointed places in the business place.
Place text at 40-50% of page, related illustrations and figures at the rest.
- 3) Leaflets
A5 size, vertical.
Distribute at the event and campaign.
Use colored papers other than white papers to attract people's attention.
Ratio of letters, illustrations, figures can be

changed depends on topics

At any hand, contents are easily understandable by most employees based on preliminary study. After at least 1 year from the start of information service, we conducted questionnaire survey to occupation group, and determine its coverage.

Among 3 study materials, material which attracted a lot of attention was POP menus. While some differences occurred in each field, about 50% of those polled answered that they read them all or pretty much with update of the contents. Especially women pay high attention as over 60%. On the other hand, only 35% of poles answered that they read all or most of posters with it's' update, and about 15% responded that they didn't read at all. And only 15% of men and 20% of women replied that they read leaflets through every time the contents renewed.

POP menus was recognized as highly effective because many of them were set to come in sight for sure, and kept interests of dining room users by changing its contents sometimes at various intervals. On the other hand, dramatic effects of posters or leaflets were not always seen. It seems there were negative impacts of limiting time and space of posting and distributing, and inconvenience of moving around to see them.

The results of this time show necessity for the select and use of adequate materials according to the situation, not by just sending out health and nutrition information unilaterally.



Point of Purchase advertising menus (POP menus)

Tips on Eating Vegetables As Much As You Can!

- Vegetables get smaller in size by cooking so that you can eat great quantity easily.
- While nutrients are lost by heating, it is actually efficient if you consume a lot.

Research Findings

Gene Mest Which Enlarges Adipose Cell

Osamu Ezaki
Division of Clinical Nutrition

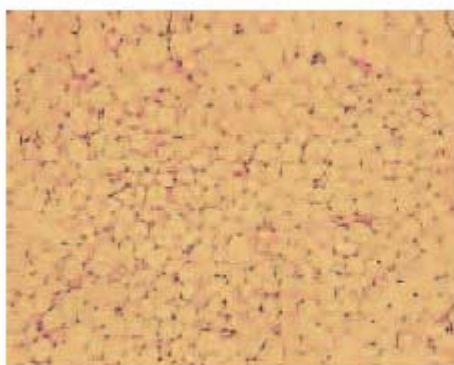
Obesity is said to be becoming a breeding ground for Type 2 diabetes, arteriosclerosis, hypertension, and hyperlipemia. Why is it so, then?

Obesity is a condition accumulating excessive body fat, not just overweight but rather a condition increasing a number of adipose cell, or a condition that adipose cell itself gets bigger. Type 2 diabetes, arteriosclerosis, hypertension, and hyperlipemia are especially developed when abdominal visceral fat cell that liver and intestines exist enlarges. Adipose cell produces various physiologically active substance or hormonelike substance as endocrine organ. These substances are generally called adipocytokines, and related to regulation of sugar metabolism or fat metabolism in the body. But enlarged adipose cell produces unusual amount of bad adipocytokine, and cause various diseases by disturbing sugar metabolism and fat metabolism.

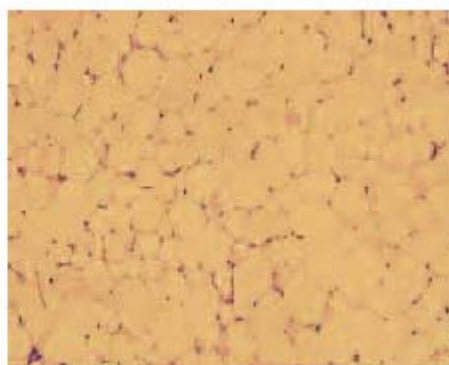
In other words, a key to exploring the cause of obesity as a breeding ground for disease is adipose cell. Then we examined mice to know what kinds of gene vary in visceral fat cell. We found that gene called Mest was significantly expressed in overweight mice by high-fat diet, or genetically overweight and diabetic mice. And we conducted various tests to know how Mest

works in adipose cell.

As a result, Mest didn't express in tissue other than adipose cell, and it only significantly expressed in adipose cell of fat mice. Obesity often accompanies abnormality in the metabolism of sugar, and Mest is deeply associated with obesity itself rather than regulation of blood sugar. On the other hand, tests on intake of pioglitazone, drug for Type 2 diabetes proved that expression of Mest reduced when enlarged adipose cell got smaller. In addition, tests on cultured cell of over expressed Mest promotes expression of specific gene including adipocytokine gene in adipose cell. We created transgenic mice that overexpressed Mest in adipose cell, and evaluated activity of Mest in the body. Then this transgenic mice increased expression of gene specific for fat, and enlarged adipose cell. Consequently, Mest came out not only in enlarging adipose cell but also increasing expression of bad adipocytokine. To put it the other way around, control expression of Mest suggests preventing enlargement of adipose cell and abnormal secretion of adipocytokine. For these reasons, creating a drug targeting on Mest may be effective to prevent obesity or various diseases that associates with this.



adipose cell of original mice



adipose cell of transgenic mice

Figure: **Enlargement of adipose cell on Mest transgenic mice**

Research Findings

Women with high BMI tend to underreport energy intake – Study for new female university students aged 18-20 years –

Hitomi Okubo

Project of Scientific Evaluation of Dietary Reference Intakes

We frequently hear contradictory comments from weight-conscious people as to “although no eating, gaining weight”, or receives answers from obese people at dietary survey like “no eating” while they must’ve been eaten. These concerns a phenomenon called ‘underreporting’ that is estimating less than amount of actual intake, and one of the problems that dietitians bother about. However, reports on this problem are mostly developed by the United States or Europe, and there is very few study for Japanese. So we evaluated reporting accuracy of energy intake among Japanese, and examined the feature of a group which likely underreport.

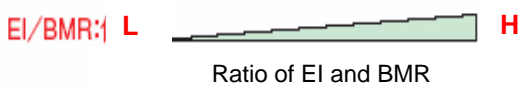
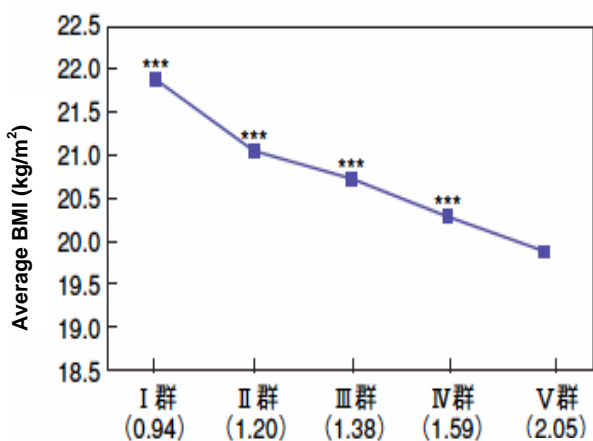
This survey was conducted based on the cooperation of female university students aged 18-20 y who entered a college in 1997. Two kinds of questionnaires were prepared, and they were asked about foods they have eaten within one month and their lifestyle. We calculated the ratio of energy intake (EI) to basal metabolic rate (BMR), EI/BMR whether their reports of energy

intake were reasonable. It indicated that the lower the EI/BMR and the lower the reports of energy intake.

In graph 1, the subjects were divided into five groups by this ratio, and compared their BMI. The lower EI/BMR group had the higher BMI. In graph 2, it shows the nutrient intake gradient according to the level of underreporting. Compared to the group V which reported the highest energy intake, the lower energy intake groups tended to report less intake of fat and protein, and more of carbohydrates. These results indicate that women whose BMI are high tend to underreport. Reporting macro nutrient intake balance may also be correlated with accuracy of reporting energy intake.

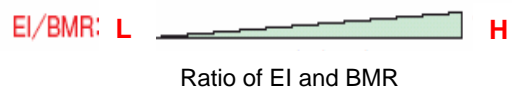
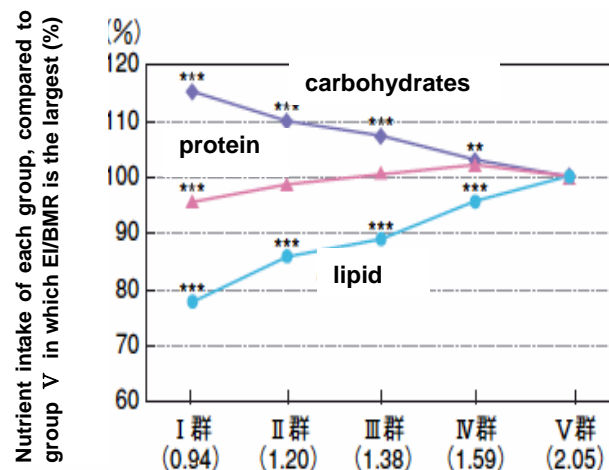
Subjects of this study are female university students aged 18-20 y, and the results may not be always true for all Japanese. However, we need to keep it in our mind that ‘underreporting’ may exist in obese people.

Graph1. Relationship between energy intake (EI/BMR) by self-assessment & BMI



Difference Test with EI/BMR of group V;
One-way analysis of variance (Dunnnett's)

Graph2. Relationship between energy intake (EI/BMR) by self-assessment & nutrient intake



Difference Test with EI/BMR of group V;
One-way analysis of variance (Dunnnett's)

Research Findings

Increasing Thinness among Young Women

Hidemi Takimoto

Center for Collaborative Research

Obesity is one of the major health problems on a worldwide scale. Not a single day passes by without hearing the news about health risks due to obesity in the mass media. We fear that too much strength on obesity prevention might lead young women who have slimming obsession to unnecessary weight loss. Excessive weight loss is pointed out as causing nutritional deficiency, irregular menstruation, and eating disorders. In developed countries, health risk of underweight is not as popular as that of overweight. But a cohort study on middle-aged and senior adults showed that the mortality of “underweight” women was higher than one of “normal” weight women. And women who were underweight before pregnancy have a higher risk of intrauterine growth retardation or premature births. Then, is there an evidence of thinness in young women increasing?

We used data on height and weight in 30,903 non-pregnant and non-lactating women aged 15-29-year-old, who participated in the National Nutrition Survey in 1976-2000, and examined change during the 25 years. Subjects were divided into 3 groups as 15-19-year-old, 20-24-year-old, 25-29-year-old, and terms were divided into 5 groups of 5 years each. We used standards of body mass index from Japan Society for the Study of Obesity, and judged BMI of less

than 18.5 kg/m² as “underweight.”

Figure shows ratio of BMI and thinness by age for 25 years. As the figure shows, significant decline in average BMI, and significant increase of thinness, can be seen in 15-29-year-old women. In particular, in a group of 25-29-year-old women, ratio of thinness increased significantly from 13.5% during 1976-1980 to 23.7% during 1996-2000. During the survey period of 25 years, growth of average height of all ages increased significantly while average weight didn't change very much.

Why did thin women increase like this? As one of the reasons of this, increase of eating disorder such as anorexia can be assumed. But frequency in Japan is estimated as 17~30 women in 100,000 women, and it is low compared to 270 women in the US and 115 women in the UK. Another reason we can think of is increase in smoking rate. From the results of national nutrition survey, smoking rate of 20-year-old women was 11.9% in 1990, and it almost doubled of 20.9% in 2000.

From now on, we need to conduct a further investigation into the reasons why thin women increased like this, and investigate health problems (anemia, irregular menstruation and so forth) that thinness women are likely be affected.

