Enzyme Wave

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Photo kyodo news

Arrival of era of Superconducting Maglev System
will open the door for significant growth in NAGOYA.

AMANO
Amano Enzyme

Enzyme Wave vol.18

CONTENTS

Linear Chuo Shinkansen

Photo kyodo news

Column

Arrival of the era of the Superconducting Maglev System will open the door for significant growth in the Tokai region

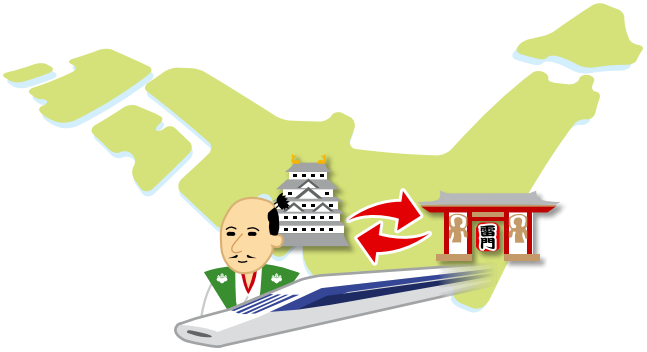
The government has approved the project to build the new "Chuo Shinkansen" magnetically levitated bullet train line linking Shinagawa, Tokyo, and Nagoya. Maglev trains, set to start commercial service in 2027, will link Shinagawa with Nagoya in only about 40 minutes, enabling easy travel between the Tokyo metropolitan area and the Nagoya metropolitan area, virtually making them one single metropolitan area. The project will mark the arrival of an era of revolutionary high-speed transportation.

By using superconductivity maglev trains can travel at a speed of 580 kilometers per hour, which is comparable to that of aircraft. This will be the first time this technology is implemented, so therefore it will be the world's fastest railway line. The high speeds achieved by maglev trains will save travel time, thereby increasing business efficiency, enhancing productivity and revitalizing the economy. The economic benefits expected after commercial maglev train service starts are estimated by our company at 10.7 trillion yen (benefits lasting 50 years). Given that this sum does not include benefits associated with urban development projects expected in the vicinity of the maglev train line, and spending by foreign tourists, greater economic benefits are expected to be generated in areas along the maglev train route.

The area reachable within two hours in central Tokyo by people coming from Nagoya using a maglev train will be significantly greater than that by using a Shinkansen Nozomi bullet train. As a result, the number of business offices in Tokyo reachable within two hours for business purposes will multiply by a factor a five. In terms of time distance, the city area surrounding Nagoya station will become part of the Tokyo metropolitan area. In the case of a maglev train, an additional fare will be charged. However, the margin above the current one-way fare of 11,000 yen charged for the Nagoya-Shinagawa route taken by a Nozomi bullet train will be only about 700 yen more. This means that a maglev train will be an economical mean of transportation that happens to be very high-speed.

Let's think about how business potential will grow in the Tokai region centering on Nagoya once the era of maglev trains arrives. The first is regarding land costs for business operations. The cost of renting business offices housed in high-rise buildings near Nagoya station is much cheaper than those near Tokyo station and Shinagawa station. Also housing costs in the Nagoya area are also significantly cheaper than those in Tokyo, thus benefiting employees in terms of living expenses. In addition, the Nagoya metropolitan area is less crowded than the Tokyo area, the time spent on commuting is shorter, and the area is located close to coastal and mountainous areas where people can spend a leisurely time. These factors enable the Nagoya area to be publicized as an attractive metropolitan area in terms of economy, time and space. The second is regarding the role of the Nagoya area as a national land asset. Three expressways (Tomei, New Tomei and Chuo expressways) have been already built between Tokyo and Aichi Prefecture. If the maglev train project is completed between Tokyo and Nagoya, the Shinkansen service will be duplicated, further increasing ground transportation options between the two cities. This will prevent the distribution of goods and flows of people between Tokyo and Aichi from coming to a complete halt in the event of a large-scale disaster. Based on this assumption, it is advisable for key city functions currently concentrated in Tokyo to be partially transferred to Aichi Prefecture a step aimed at diffusing the risk of city functions being paralyzed in the event of a disaster, which is currently fairly significant due to the concentration of functions in the Tokyo metropolitan area. Under these circumstances, Nagoya is likely to be regarded as an important area for both Japanese and foreign companies in terms of effective use of land.

There is however a fear that the scheduled start of maglev train services will relocate human resources and capital from the Nagoya metropolitan area to the Tokyo metropolitan area, resulting in the decline of the Nagoya area. Even so, the new train service should still be regarded as an opportunity for further growth and development in the Nagoya (Tokai) region. It is hoped that during the 12 years preparation period until the service start of maglev trains in 2027, both the public and private sectors in the will work together in order to maximize the economic benefits of the project while also using this period as a springboard for achieving significant growth for the regional economy in the future.



Yoshito Kato

Deputy Division Head ,Principal, Policy Research & Consulting Division
Mitsubishi UFJ Research and Consulting Co., Ltd.

【Background】

Hailing from Gifu City, Mr. Kato entered Nomura Research Institute in 1987. He moved to Tokai Research & Consulting Inc., the predecessor of Mitsubishi UFJ Research and Consulting Co., in 1995. He remained with Tokai Research & Consulting, which later became Mitsubishi UFJ Research and Consulting, through a merger with two other think tanks, until taking up his current post. His areas of specialty are research and consulting related to economic benefits from the establishment of social infrastructure, and regional development and fund raising. Economic benefits from the establishment of social infrastructure analyzed by Mr. Kato include benefits from road, airport and port projects, and more recently cover the effects expected from the "Chuo Shinkansen" maglev train project. He is also engaged in work related to PFI and PPP, with his expertise covering economic and financial matters involving public works projects. Mr. Kato concurrently serves as a guest professor at Gifu University.

Symposium

Holding Amano's 15th Symposium on Enzyme Applications

On June 13, 2014, a fine sunny day, Amano's 15th Symposium on Enzyme Applications was held at Amano Jizendo Hall, located on the site of Amano Enzyme's former Nishiharu Plant. At this 15th memorial meeting, in addition to five conventional research encouragement awards, one award was provided for a research project in the medical and pharmacological field that was selected from the researches recommended by Professor Emeritus Tadashi Takeuchi, of Tokyo Women's Medical University. Thus, in total, six award lectures were given in the meeting. Moreover, three lecture programs and one report prepared by Amano Enzyme Inc. were also delivered.

(Affiliations at time of Awards & Lectures)

Award Lecture

Design of thermostable enzyme based on evolutionary information
Satoshi Akanuma(Tokyo University of Pharmacy and Life Sciences)

Functional analysis of chitin degradation-related enzyme producing functional oligosaccharide in bacteria
Takako Hirano(Nihon University)

A study of drug development using natural resources based on target enzyme guided synthesis using triazole formation
Tomoyasu Hirose(Kitasato University)

Addition of pyrophoshoric acid utilization to acetate kinase to increase productivity of acetyl phosphoric acid for construction of ATP regeneration system
Shigeyuki Kawai(Kyoto University)

Elucidation of diverse catalytic functions of cytochrome P450 and their application
Kenji Watanabe(University of Shizuoka)

A study of optimal use of pancreatic enzyme medicine
Atsufumi Matsumoto (Hirosaki University／Hirosaki Municipal Hospital)

Special Lecture

The birth of the oldest ecosystem on earth and challenge for an experiment regarding artificial metabolism evolution (project collaborators wanted)
Ken Takai(Japan Agency for Marine-Earth Science and Technology)

Opportunities in Halal Economy
Iswarni Salleh (General Manager, Halal Industry Development Corporation)

Power of imagination
Tetsuro Matsuzawa (Kyoto University／Japan Monkey Centre)

Reporting Lecture

Transglucosidase: functional modifications and future development
Satoru Ishihara(Amano Enzyme Inc.)

In expectation of contributions to the promotion for the utilization of enzymes, Amano Enzyme Inc. grants its encouragement awards for enzyme application researches that are highly likely to influence the industry and provides the award winners with the opportunities to give lectures at the Symposium of Enzyme Applications. The symposium, which offers its participants prime opportunities to obtain the latest knowledge about enzyme applications, serves as a forum for researchers belonging to universities and companies. We sincerely hope you will be able to attend.

Development of an oligosaccharide-producing enzyme as a dietary supplement (Part 2)

The westernization of the Japanese diet makes it difficult for many people to consume a sufficient amount of fiber for health maintenance. In an effort to develop a method for easy fiber intake, we undertook a study to utilize an enzyme-transglucosidase-as a dietary supplement to produce oligosaccharides in the digestive tract. In Enzyme Wave No. 1, we reported that oligosaccharides were actually produced from food in the digestive tract in response to the intake of the oligosaccharide-producing enzyme, transglucosidase. Moreover, an animal experiment demonstrated that production of oligosaccharides cancelled energy intake, which resulted in better weight control. In Enzyme Wave No. 12, we introduced the effect of transglucosidase as a dietary supplement to control blood glucose levels in healthy volunteers and discussed its ability to suppress inflammation in the intestinal tract in a mouse enteritis model (*J. Clin. Biochem. Nutr.* 2007; 41 (3): 191-6.).

Development of an oligosaccharide-producing enzyme as a dietary supplement for prevention of metabolic syndrome

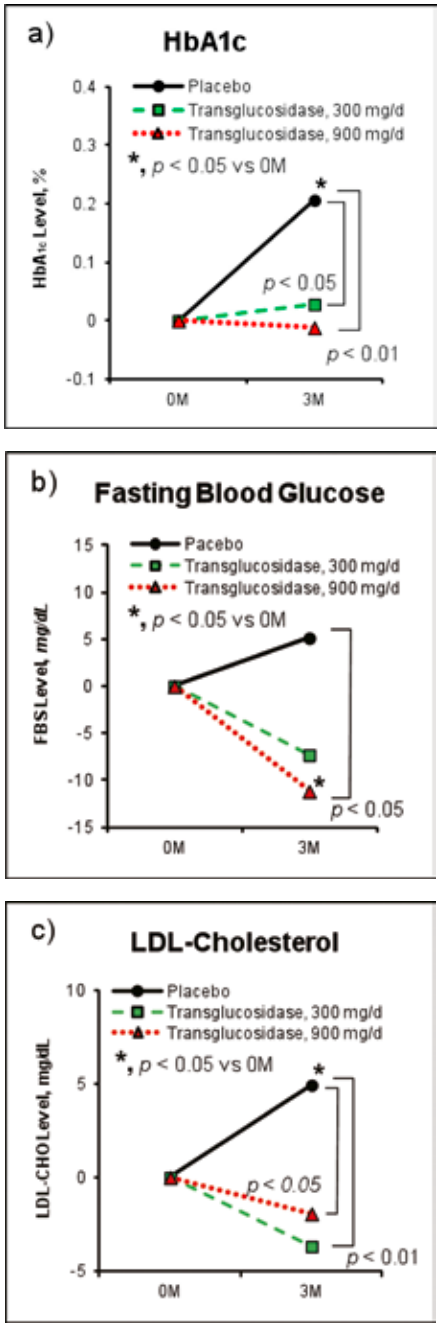
– Improvement of metabolism in patients with diabetes mellitus –

A randomized double-blind placebo-controlled study was conducted in 64 patients with type 2 diabetes mellitus (37 males, 27 females, mean age 63.8±8.4 years) (*Diabetes Obes. Metab.* 2012; 14 (4): 379-82.). Nineteen patients were assigned to the placebo group, 23 patients to the transglucosidase 300 mg/day group, and 22 patients to the transglucosidase 900 mg/day group. Their data on blood glucose, lipids, high molecular weight adiponectin, liver function, body weight and blood pressure were collected before treatment and 12 weeks after initiating treatment, and the pre-treatment and post-treatment data were compared among the groups.

First, HbA1c, the mean blood glucose level, improved in 18.8% of patients in the placebo group, 33.3% in the transglucosidase 300 mg/day group, and 47.1% in the transglucosidase 900 mg/day group: the number of patients who showed improved HbA1c increased remarkably after transglucosidase administration. HbA1c values increased considerably in the placebo group, while HbA1c values did not change in the two transglucosidase groups; thus, transglucosidase significantly suppressed the HbA1c value increase (Fig. 1a). Compared with the fasting blood glucose level in the placebo group, the higher-dose transglucosidase group showed a marked decrease in glucose level with a much lower post-treatment fasting blood glucose level than the pre-treatment level (Fig. 1b). Reduced blood insulin levels and improved insulin resistance (HOMA-IR) were also observed in this group. Based on these results, transglucosidase is expected to contribute to blood glucose control in patients with diabetes mellitus.

Transglucosidase also showed an effect on lipids. Low-density lipoprotein (LDL) cholesterol levels increased substantially in the placebo group, while it decreased to some extent in the transglucosidase groups. Compared with the placebo group, LDL levels in the two transglucosidase groups decreased significantly (Fig. 1c).

Figure 1



Development of an oligosaccharide-producing enzyme as a dietary supplement (Part 2)

The post-treatment triglyceride levels in the lower-dose transglucosidase groups were significantly lower than the pre-treatment levels, and the triglyceride levels in both transglucosidase groups decreased much more than they did in the placebo group (Fig. 1d).

High molecular weight adiponectin, which is reported to have an anti-atherogenic effect, significantly increased after administration of low-dose transglucosidase (Fig. 1e). This result suggests that transglucosidase might be used to control lipid metabolism and adipose cells and to exert an anti-atherogenic effect.

Although a trend of weight gain was observed in the placebo group, no weight change was seen in the transglucosidase groups (Fig. 1f). Systolic blood pressure did not change among the three groups although diastolic blood pressure increased markedly in the placebo group. Compared with placebo, high-dose transglucosidase appeared to control diastolic blood pressure with minimal increase pre- to post-treatment (Fig. 1g). While the values of ALT, AST, and GCT in the liver function tests increased significantly in the placebo group, they did not change in the transglucosidase groups. This is probably because transglucosidase increases high molecular weight adiponectin, which improves glucose/lipid metabolism and exerts an anti-TNF-α effect. By increasing high molecular weight adiponectin, transglucosidase can control the aggravation of fatty livers.

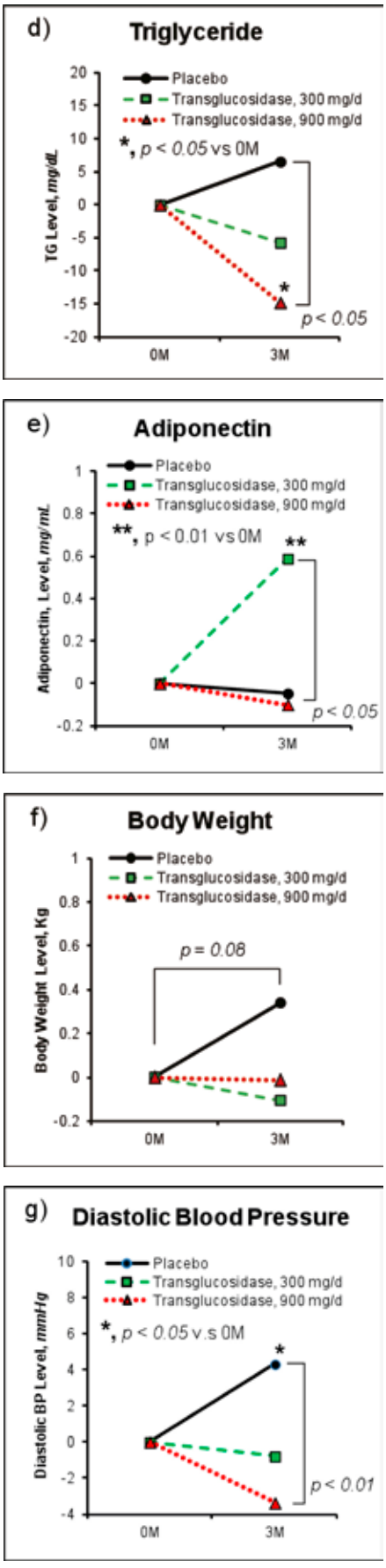
Accordingly, transglucosidase is effective in regulating metabolism as demonstrated by beneficial effects such as control of blood glucose, and improvement of insulin resistance and lipid metabolism. These observations suggest that transglucosidase may control obesity and arteriosclerosis and regulate blood pressure. Therefore, transglucosidase is expected to contribute to the prevention of cardiovascular events resulting from metabolic syndrome and the reduction of mortality associated with cardiovascular events.

■Approach to clarify the mechanism targeting human gut microbes

Recent studies propose a possible relationship between human gut microbes and various diseases including obesity and diabetes mellitus and suggest that gut microbes may play a central role in vital human activities. A comparison of gut microbes was then made between patients with diabetes mellitus and healthy volunteers. In addition, the influence of transglucosidase on the gut microbes of patients with diabetes mellitus was evaluated.

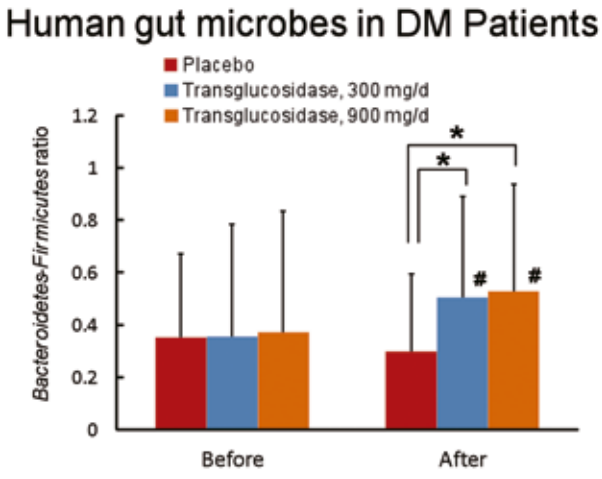
Gut microbes collected from 60 patients with diabetes mellitus (35 males, 25 females) were compared with those collected from 10 healthy volunteers (7 males, 3 females) by the terminal-restriction fragment length polymorphism (T-RFLP)method. A placebo and transglucosidase(300mg/day,900mg/day) were administered for 12 weeks to each of 20 patients with diabetes mellitus to evaluate the change in gut microbes (*BMC Gastroenterol.* 2013; 13: 81.).

Figure 1



Transglucosidase administration significantly increased the *Bacteroidetes-Firmicutes* ratio. No such increase was observed in the placebo group (Fig. 2).

Figure 2



#$p < 0.05$ vs Before, *$p < 0.05$ vs Placebo

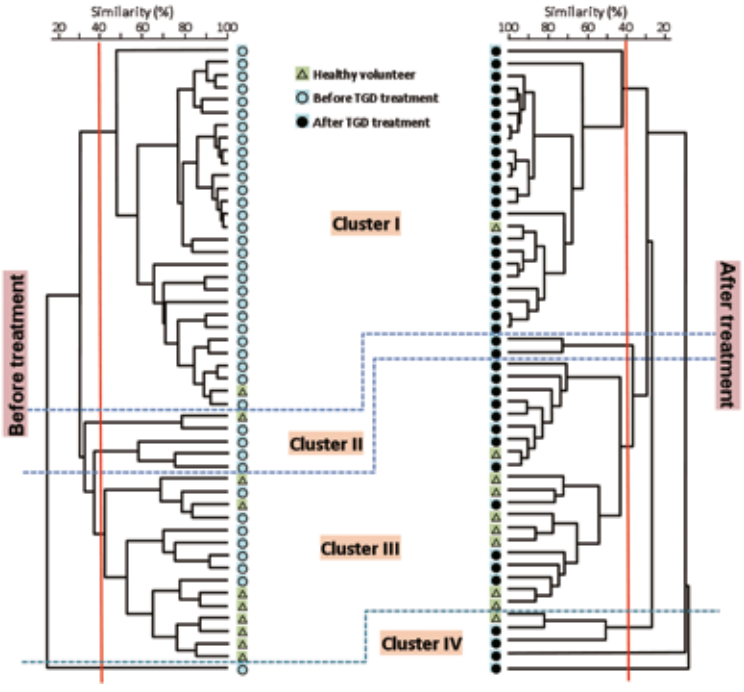
(BMC Gastroenterol.2013 May 8;13:81)

Pre-treatment and post-treatment dendrograms in the transglucosidase group(n=40) and healthy volunteer group (n=10) were prepared for comparison. Healthy volunteers were generally classified in Cluster **III**, which indicates that healthy volunteers and patients with diabetes mellitus belong to different clusters before administration of transglucosidase. Three months later, more diabetes patients were classified in Cluster **III**, suggesting that administration of transglucosidase might normalize gut microbes in patients with diabetes mellitus(Fig. 3).

Future prospects

Transglucosidase enables production of oligosaccharides from food in the digestive tract. The present study demonstrated that this method can serve fully to develop a dietary supplement for prevention of metabolic syndrome. This transglucosidase functions may be related to gut microbial population. More effort should be directed toward clarifying a detailed molecular biological mechanism in addition to the previous report on enteritis prevention mechanism.

Figure 3



Professor Makoto Sasaki

Graduation from Nagoya City University Medical School in 1989. Researcher at Louisiana State University School of Medicine in 2000. Associate professor at the Department of Gastroenterology, Aichi Medical University School of Medicine in 2009. Professor at the Department of Gastroenterology, Aichi Medical University School of Medicine in 2013. Specialty: Digestive organ disease studies.
Received the Japanese Society of Digestion and Absorption Award for his research titled "Digestion Absorption Mediated Metabolic Control by Oligosaccharide Synthesizing Enzymes – Application to Treatment/Prevention of Metabolic Syndrome."

Passing down the taste for Dashi to the next generation

Collecting and drying Rishiri kelp (photographed on Rebun Island by Fushiki)

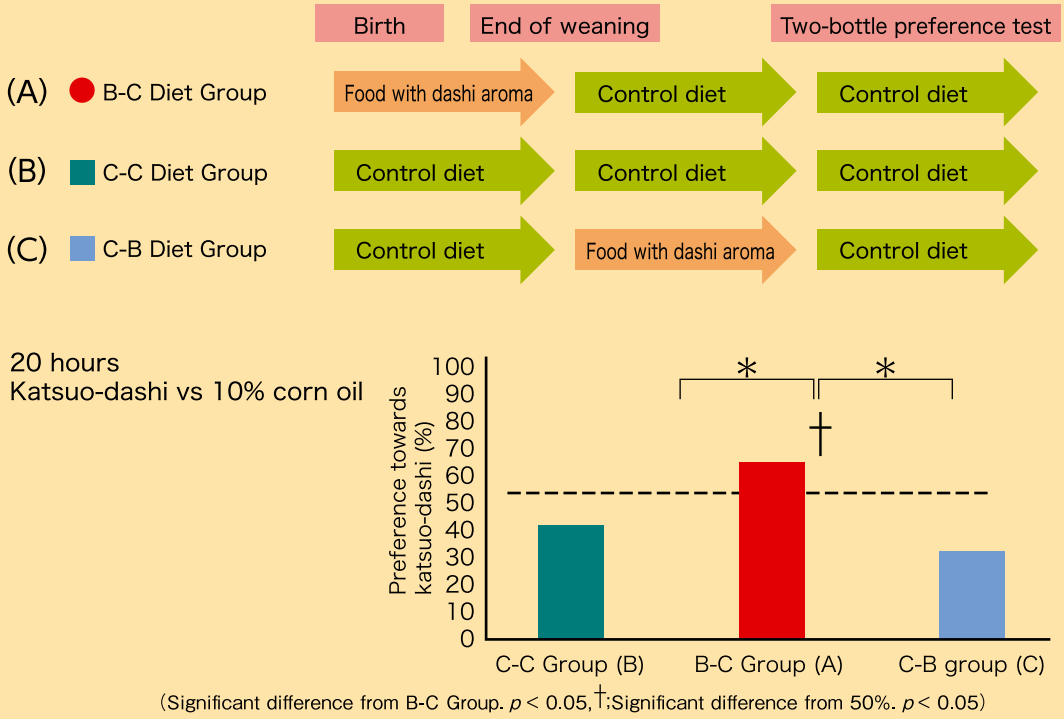
Dashi is a traditional and wide-spread soup (or soup stock) used in various Japanese cuisines. It is made from edible kelp, known as kombu, or katsuo-bushi (dried bonito), shaved fish flakes of fermented skipjack tuna). The taste of dashi can be described as a combination of umami, which is recognized as a type of taste, and the aroma which seeps into the nose from the oral cavity, known as fuumi. The main means of enjoying the fuumi is as an olfactory sensation. A sophisticated dashi fuumi boosts the taste to a higher dimension, so that the senses of taste and smell cannot be separated. It is often said that the taste of a perfect dashi comes in various forms such as long lastingness, thickness, spread, mildness and richness, all at the same time.

Umami is considered one of the tastes we innately favor and globally accept, like sweetness and the taste of oily foods. Japanese kelp, especially, contains a great amount of glutamic acid, enabling us to obtain a solution of a highly concentrated and pure umami just by soaking some Japanese kelp in cold or hot water (Figure 1). In addition, the nucleic acid in katsuo-bushi can drastically increase umami.

On the other hand, the fuumi of dashi or soup comes from the aroma constituents found in the ingredients, which are extracted together with the umami. Since dashi is made by extracting umami out of locally grown food materials, and because ingredients vary in each region and country, the fuumi of dashi is different in each locale. Although umami is innately favored, the preference for fuumi is however acquired, so it is learned from previous eating experience. So, a type of fuumi which was not previously present in a eating culture, is not so easily accepted. To like dashi to have accepted the aroma of the regional food materials used in making it. Olfactory memory remains unchanged for a long time. Even the memory of a smell that is decades old can stay very clear. Humans have more than 400 kinds of olfactory receptors, so the patterns of response are countless. Only through fuumi, as in by sense of smell, can we distinguish the subtle differences of different foods. Meanwhile, the sense of taste is not so refined and thus one's memory of a taste is often unclear. The preference which is passed down from culture is actually the memory of fuumi (smell).

Many reports on how food preference is based on experiences in the weaning and infant periods exist. Our study group conducted experiments with animals to investigate how dashi that people eat in their childhood can influence their preferences when they are adults. Mice were divided into three groups: (A) first, those receiving katsuo (bonito)-dashi from their parents' generation all the way to completion of weaning, (B) those receiving no katsuo-dashi at all from their parents' generation all the way to becoming adults (the control group), and (C) those receiving katsuo-dashi after completion of weaning. When given a choice between bottle containing water or one

Katsuo-dashi experienced prior to the end of weaning shows a greatly increased preference towards katsuo-dashi at adulthood



(Significant difference from B-C Group. $p < 0.05$, †;Significant difference from 50%. $p < 0.05$)

Figure 1: Exposure of mice to katsuo-bushi fuumi in their weaning period increased the level of their preference to katsuo-bushi fuumi. Source: Fushiki

with a katsuo-dashi solution, all three groups showed preference towards the katsuo-dashi one. On the other hand, when a 10% solution of corn oil was tested against katsuo-dashi, only the (A) group of mice who received katsuo-dashi before the end of weaning was observed to have preferred katsuo-dashi more than corn oil (Figure 2). This research indicates how the experience of eating katsuo-dashi in the periods before or after weaning can strongly influence one's taste preferences.

When it comes to children, two elements appear to be important in establishing taste preferences: one is creating for them a nutritious environment during infancy and childhood; and the other is establishing proper networks before the age of ten. Once weaning ends, the period of time before children can eat adult food is quite long. Therefore, it seems that a long-time experience of eating dashi starting from infancy, during kindergarten, and all the way through elementary school may influence their preferences later.

The amount of rice eaten by people in Japan is today half that of previous years. In addition to rice, consumption of other traditional Japanese foods such as miso (soy paste), soy sauce, and mirin (sweet sake used for cooking) have also decreased. The preservation of traditional foods such as dashi is deeply connected to the consumption of rice, suggesting that a preference for dashi can be maintained only by smoothly passing down traditional Japanese foods to the next generation.

Professor Tohru Fushiki

Born in 1953 in Kyoto Prefecture, Japan. Graduated from the Department of Food Engineering, Faculty of Agriculture, Kyoto University in 1975, and completed the doctoral program at the Graduate School of Agriculture, Kyoto University in 1980. After being Assistant Professor and Associate Professor at Kyoto University, he was professor at the Graduate School of Agriculture at Kyoto University from 1994 to March 2015. Since April 2015, he has been a professor at Ryukoku University. He specializes in food biochemistry and nutrition chemistry. He also serves as a board director for the NPO Japanese Culinary Academy and is actively involved in community-based food education programs to pass on the food culture of Japan to future generations. He also is involved in activities to disseminate umami, the basis of many dishes in Japanese cuisine, to chefs of the next generation, not only in Japan, but around the world as well.

In addition to academic papers in his specialized fields, he has published many books including.

Topic

JOKICHI TAKAMINE, Father of Modern Biotechnology

Dr. Jokichi Takamine
(photo courtesy Great People of Kanazawa Memorial Museum)

Dr. Jokichi Takamine (1854–1922)

Dr. Takamine, who lived during the turbulent years of the late Edo, Meiji, and Taisho periods, made great achievements as a scientist, an entrepreneur, and a contributor to friendly international relations. In the process of developing a technique for whisky making by applying the Japanese method of alcohol brewing and fermentation based on *koji*, a fungus widely used in Japan, he managed to isolate a powerful digestive enzyme, later named takadiastase, and then developed it into a pharmaceutical product. Dr. Takamine also succeeded in isolating and crystallizing the hormone adrenaline from cow adrenal glands. This discovery greatly contributed to the world's medicine and pharmacology fields, giving rise to neuroscience and endocrinology.

The two major discoveries cemented Dr. Takamine's status in the United States, and his subsequent research and development relating to microbial starch-decomposing enzymes centering on takadiastase earned him the title of " Father of Modern Biotechnology."

Besides scientific research, he advocated the establishment of a national institution for chemical research to train young Japanese researchers abreast with scientific advances in the world. This was realized in the establishment of the Institute of Physical and Chemical Research (RIKEN). In the field of international exchange, he is known to have planned and financed the planting of cherry blossom trees along the Potomac River in Washington D.C. He has also donated cherry blossom trees to the city of New York.

The Jokichi Takamine Research Foundation, specified nonprofit corporation

The Jokichi Takamine Research Foundation aims at making widely known the life and work of Dr. Takamine, who continues to serve as a great model through his contributions to the advancement of science, application of scientific achievements in business development, and reinforcement of the US-Japan friendship in modern Japan. The goal is to entice the public to learn from this great man and excel in turn in their respective fields, especially in inspiring and encouraging young people to embrace their hopes and dreams as future leaders. Accordingly, the foundation continues to collect information on Dr. Takamine, regularly publish a journal, and organize lectures and other events to widely inform the public of Dr. Takamine's achievements.

Major Past Activities

The foundation has participated as a speaker and/or exhibitor at various international scientific conferences and trade fairs, including ifia JAPAN 2011 (the International Food Ingredients & Additives Exhibition and Conference) and the International Union of Microbiological Societies (IUMS 2011). Each year (2014 was the 7th times) the foundation has also organized lectures at municipal lower secondary schools in Takaoka City, Toyama Prefecture, also known as Dr. Takamine's birthplace, and other parts of Japan as well. The foundation also provides information and materials to newspapers, TV stations, magazines, and other media.

Seeking New Members

Members of the foundation will receive the regular bulletin, along with publications relating to Dr. Takamine, information on activities, such as lectures and events, as well as media coverage (newspaper, TV, magazine, and other) on Dr. Takamine, and any newly acquired information relating to him.

To become a member, please write to the address below with your name, address (including postal code), company and department name (if a corporation), telephone number (fixed line), occupation, age, and gender. You will then be sent instructions and forms for admission and annual membership fees.

Secretariat, The Jokichi Takamine Research Foundation
Kyoyu Bldg., 5/F, 3-12-5 Akasaka, Minato-ku, Tokyo 107-0052, Japan
* Details (in Japanese) are also viewable on our website:
http://www.npo-takamine.org/ask.html

Lecture and exhibition at IUMS 2011

Mini-lecture in front of Takamine Shrine, home of Tanba sake brewers

Lecture at a lower secondary school in Takaoka City

Documents for members

Application of Enzymes to Infertility Treatments and Assisted Reproductive Technologies

Introduction

Advanced infertility treatment technologies have been developed in Japan. The number of persons who undergo infertility treatments including in-vitro fertilization (IVF) is increasing rapidly. In addition to male and female reproductive dysfunction, lifestyle changes and delayed marriage have been pointed out as complex factors behind this rapid increase. In the present study, infertility is examined, and the present conditions of and representative treatments for infertility are explained. Moreover, the application of enzymes in infertility treatments is discussed.

Infertility

Infertility is defined as the failure to achieve a clinical pregnancy after a certain period of regular unprotected sexual intercourse. Generally, "a certain period" indicates two years in Japan (one year according to the WHO's definition). However, even if the period of regular unprotected sexual intercourse is less than two years, a diagnosis of infertility is often made in the following cases: the possibility of pregnancy is low after timely sexual intercourse or both partners are 30 years old or older.

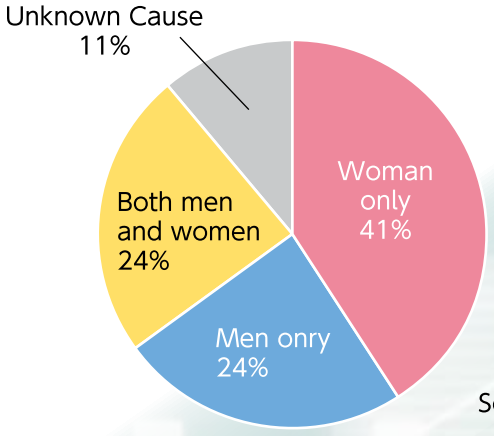
Infertility results from either male or female reproductive dysfunction or from both male and female reproductive dysfunction together. The main causes of female reproductive dysfunction are ovulation disorders including ovarian dysfunction, tubal stenosis, tubal adhesion, and uterine fibroids. The main causes of male reproductive dysfunction are sexual dysfunction such as erectile disorder and azoospermia. According to the WHO's statistical data, infertility due to female dysfunction alone accounts for 41%, while infertility due to male dysfunction alone accounts for 24%. Infertility due to both male and female dysfunction accounts for 24%. (See the pie chart below.)

In addition to reproductive dysfunction, age is regarded as a factor that can prevent pregnancy. The frequency of infertility is below 10% in women aged 29 or younger. This frequency exceeds 20% in women over the age of 35 and increases nearly up to 30% in women ranging from 40 to 45 in age [*1]. As a result of recent social changes, the female mean first-marriage age and female mean age at first birth are increasing. Women first gave birth at around 27 years of age 20 years ago but at around 30 years of age more recently [*2]. Age-related infertility is expected to increase if the female first-marriage age and female age at first birth continue to rise in the future.

Infertility treatments

Infertility treatments are artificial therapeutic technologies that successfully promote the process from insemination to pregnancy and delivery. They are technically known as "auxiliary reproductive medicine." Infertility treatments are broadly divided into general infertility treatments and assisted reproductive technologies (ARTs). The representative treatments included in these categories are listed in the table below.

Causes of infertility



Source : WHO Survey

Application of Enzymes to Infertility Treatments and Assisted Reproductive Technologies

In most cases, the infertility treatment starts with general infertility treatments, either the timing method or drug therapy. The treatments classified in this category are covered by insurance, and one treatment session costs several thousand yen. If these treatments prove to be ineffective in achieving pregnancy, the subsequent treatments are introduced. Artificial insemination is selected to treat male reproductive dysfunction (erectile disorder, azoospermia). The fertility rate could be as low as about 8% but one treatment costs about 10,000-20,000 yen. This treatment is advantageous because it does not impose a big financial burden on couples.

IVF, a type of ART, is used in case of failure to accomplish in-vivo fertilization. In the IVF process, sperms are added to the culture containing eggs collected before ovulation, and the culture is observed until natural fertilization is achieved. After repeated cell division in the culture, the fertilized egg is examined if its condition is satisfactory. Then the fertilized egg is transferred to the uterus.

Sperm fertilizing ability is one of the important conditions for IVF. As mentioned, IVF is achieved outside of the human body but this method depends on the fertility of the egg and fertilizing ability of the sperm. Even if a woman is capable of conception, her partner may have a problem with sperm (problem of sperm concentration or motility). In this case, the couple cannot achieve pregnancy through IVF. If the man has a problem with sperm, the couple may choose microfertilization.

Like IVF, microfertilization is an in-vitro technique. Eggs and sperms are removed from the human body for fertilization in a laboratory. Microfertilization, however, is distinctly different from IVF because fertilization does not depend on the sperm concentration or motility in microfertilization. After being removed from the human body, the sperm is collected with a microneedle for direct injection into an egg under microscopy (intracytoplasmic sperm injection: ICSI). Then, as in the case of IVF, the satisfactorily fertilized egg is transferred to the uterus.

Present condition of ARTs

According to 2010 data published by the Ministry of Health, Labor and Welfare [*4], the number of users of ARTs in one year was about 242,000 in Japan and about 147,000 in the U.S. Unlike general infertility treatments, ARTs require special facilities and technical capabilities, and a session of treat-

	Treatment method	Treatment details	Social insurance	Cost/session*3
General infertility treatments	Timing method	Method of estimating the day of ovulation and promoting natural pregnancy. Guidance is the main part of treatment.	Covered	~Several thousand yen
	Drug therapy	Treatment with ovulation-inducing drugs.	Covered	~Several thousand yen
	Artificial insemination	Direct injection of the semen into a female's uterus for the purpose of assisting pregnancy.	Not covered	10,000 yen and over
Assisted reproductive technologies	In-vitro fertilization	Therapeutic process of natural fertilization by combining a collected egg and sperm in a laboratory dish. After in-vitro culture for a few days, the embryo is transferred to the uterus.	Not covered	200,000 yen and over
	Microfertilization	Sperm in a microneedle is injected directly into an egg under a microscope. As in the case of in-vitro fertilization, after in-vitro culture, the embryo is transferred to the uterus.	Not covered	200,000 yen and over

Based on the survey done in February 2015

ment costs 200,000-500,000 yen or more. Because ARTs do not assure 100% fertilization rate, many patients receive repeated ART-based treatments that impose considerable financial burden on them. In 2004, to reduce treatment-related financial burdens, the Japanese government and designated prefectures/cities added ART-based treatments to the list of treatments under the special treatment support program. Since then, they have partially supported ART-based treatment costs.

The number of users of this support program rapidly increased from about 18,000 at the beginning to about 135,000 in 2012, representing about an eight-fold increase [*5]. In addition to this support program, growing awareness and penetration of infertility treatment and the trend of bearing children at a later age contribute to the rapid increase in the number of users of this support program. This overall trend is predicted to continue in the future.

With the increased number of applications for financial support, the support program is being reviewed periodically. In 2016, persons who can apply for this financial support and details about this support program will be revised. Currently, people are allowed to use the support program twice a year for five years. In the new support program, however, persons having infertility problems can use it without limitation. We expect the environment of infertility treatments including ARTs will continue being improved.

Application of enzymes to ARTs

Among various infertility treatments, microfertilization is the only treatment that is based on the enzyme hyaluronidase. A collected egg is surrounded by hyaluronic acid-rich cumulus cells that enable it to adhere to the fallopian tube wall. During microfertilization, these cumulus cells prevent a microneedle from being inserted into the egg. By treating the egg surrounded by cumulus cells with hyaluronidase, the cumulus cells are eliminated within one minute.

Because of the selective specificity of hyaluronidase, the enzyme acts only on cumulus cells. Hyaluronidase is thus highly unlikely to damage the egg itself. Hyaluronidase was first used in microfertilization around 1992. Since then, it has been used globally in clinical settings [*6].

Enzymes are not used in the remaining infertility treatments. Currently, many researchers study various infertility treatments to increase the fertilization rate. They are expected to find further methods that utilize the specificity of enzymes. To utilize enzymes in infertility treatments, having an enzyme product design that meets quality requirements seems to be indispensable.

Conclusion

Each year, an increasing number of persons undergo infertility treatments. Aiming to cope with the declining birth rate, the Japanese government provides additional support for ARTs. Currently, about 3.7% of children born in Japan are done so through the use of ARTs [*7]. For couples who desperately want children, ARTs are being positioned as a key option that can bring rays of hope.

Due to space limitation, complete information cannot be covered in this article. Even if couples receive these treatments, the pregnancy success rate remains at slightly below 35%, and the success rate of delivery remains at the 10% level. As these data show, infertility is actually a very difficult problem. Further improvement of treatment technologies is indispensable, and the ethical and safety problems remain to be solved. The Japanese government and research institutes are expected to make continuous efforts to improve this difficult situation.

<References>

*1:Japan Society for Reproductive Medicine

*2:Ministry of Health, Labor and Welfare

*3:"How much do fertility treatments cost?"Asada Ladies Clinic, etc., Toyo Keizai Online.(in Japanese)

*4,*5:Ministry of Health, Labor and Welfare,Report prepared by the "Committee to Discuss the Details of the Special Treatment Support Programs for Persons with Fertility Problems" (Reference material)

*6:Fertility treatment and child intelligence, attention, and executive functions in 5-year-old singletons, a cohort study. Epidemiology Section, School of Public Health, Aarhus University, Aarhus, Denmark.

*7:"Mystery of science". Nihon Keizai Shimbun, Morning Edition, October 19, 2014. (in Japanese)

Active Enzyme Molecule 2014

Active Enzyme Molecule 2014 was held three days from December 17, 2014 in Toyama, Japan. The lecturers including around world guest speakers gave various interesting presentations including the keynote speech and scientific lectures for this conference. They also reported a large variety of findings obtained by using enzymes produced by integrating organic chemical technology and enzyme engineering technology. These new enzymes will be applied to mass exchange, new enzyme screening and production of medical/pharmacological supplements.

(Center) Yufeng Miao Dr. won the Amano Award
(right) Scheneider professor of Jury

Amano Enzyme established the Amano Enzyme Inc. Award to support young researchers. The Active Enzyme Molecule(AEM) Award Review Committee consisting of President professor Schneider and 6 members reviewed the presentations focusing on novelty of enzyme, scientific contents, and clarity of presentation, and granted to Dr. Yufeng Maio's (University of Groningen, The Netherlands) presentation titled 'Asymmetric Michael-type additions of aldehydes to nitroolefins by 4-oxalocrotonate tautomerase(4-OT) yielding valuable precursors for GABA-based pharmaceuticals.'

Conference presentation

In 2014 Amano Enzyme has decided academic presentations such as the following.
Please look forward to the activities of the future of Amano Enzyme.

Conference / Meeting	Date	Title	Speaker/ Presenter
Gordon Research Conferences Biocatalysis 2014 (Smithfield, USA)	Jul. 5~11, 2014	Molecular modifications for α-glucosidase of *Aspergillus niger,* show the altered substrate apecificity and strong transglycosylation activity	Satoru Ishihara, Satoshi Koikeda
Japan Digestive Disease Week 2014 (Kobe, Japan)	Oct. 25, 2014	Comparison of digestive activities of digestive enzyme preparations containing pancreatin	Manabu Kuroda, Shigeru Ko
45th General Meeting of The Japanese Society of Digestion and Absorption (Tokyo, Japan)	Nov. 22, 2014	Digestive enzymes for medicine - Visiting old, Learn new- (Amano Enzyme Sponsored Luncheon seminar)	Yusuke Tando, Shigeru Ko, chaired by Mitsuo Nakamura
The First Myanmar-Japan Symposium (Pathein, Myanmar)	Nov. 30, 2014	The enzyme utilization and advantage, using with microorganisms	Kensuke Yuki

From our Historical Archive of Enzymology

— Dr. Tonegawa's Research Activities and Enzymes —

Amano Enzyme's Historical Archive of Enzymology, which was established in June 2010, contains more than just books.

The archive has a book titled "Sprit and substance". This book is based on an interview between Dr. Susumu Tonegawa, who won the Nobel Prize for Physiology or Medicine in 1987 for his discovery of the "genetic principle for generation of antibody diversity", and Takashi Tachibana, a well-known journalist in Japan. The discovery is said to be highly appreciated as a once-in-a-century breakthrough achievement.

Animals have several defense mechanisms for the elimination of external intruders, one of which is the defense system called antigen-antibody reaction. Once an antigen invades the living body, it is first recognized then memorized, and on subsequent intrusion a protein to counteract the previously encountered antigen is created so it can then begin to detoxify it.

As outlined in the central dogma of molecular biology, proteins are synthesized by ribosomes according to RNA which is first transcribed from a gene DNA that carries genetic information. Beadle and Tatum's experiments gave rise to the "one gene-one enzyme hypothesis" which states that one gene has the information to create one protein, and it was also understood that one antibody binds specifically to one antigen. However in spite of this the fact remained that although human beings were at that time estimated to possess only a few tens of thousands of genes (the figure is currently estimated to be around 22,000), antibodies (proteins) were being produced that were capable of counteracting millions, and even tens of millions of antigens.

This contradiction was an immunological mystery in the 1970s when Dr.Tonegawa started his research at the Basel Institute for Immunology in Switzerland. Dr.Tonegawa attempted to solve this mystery using the new technology based on the "Restriction Enzyme" that he had fostered at the Salk Institute in the U.S. Various bacteria, such as Streptomyces and Cyanobacteria, have restriction enzymes that cleave and knock out the DNA of invading viruses such as bacteriophages. Using this technology, Dr. Tonegawa demonstrated that genes of animals, which were believed to be unchangeable, are indeed modified through recombination throughout the process of maturation which animals experience during early life. Thanks to this scientific achievement, we have come to know how animals are able to counteract external intruders by producing specific antibody proteins which can bind to all the numerous antigens they encounter.

During his interview Dr. Tonegawa explains that "science is just like manual labour," referring to the many hours he spent toiling over the preparation of the just discovered "restriction enzyme" in order to obtain targeted DNA fragments.

Amano Enzyme Message Board (May - December 2015)

Exhibition Information

Please come to our booth!
We look forward to seeing all of you from around the world.

Date	Exhibition	Location
May 20~22, 2015	ifia JAPAN 2015	Tokyo, Japan
June 24~26, 2015	CPhI China 2015	Shanghai, China
July 11~14, 2015	IFT 2015	Chicago, USA
September 9~11, 2015	FiAsia 2015	Bangkok, Thailand
October 5~9, 2015	Supply Side West 2015	Las Vegas, USA
October 13~15, 2015	CPhI Worldwide 2015	Madrid, Spain
December 1~3, 2015	Fi Europe & Ni 2015	Paris, France

Amano Enzyme participation status at 2014 exhibisions

During 2014, Amano Enzyme exhibited at various pharmaceutical and food industry-related trade shows at several locations around the world.
(Japan, China, Indonesia, Australia, France, and the U.S.A.).

Hello Chipping Norton from UK

Amano Enzyme Europe Ltd. is situated in the rural area known as the Cotswolds in Chipping Norton, England (the official name of the UK is the United Kingdom of Great Britain and Northern Ireland. Great Britain consists of England, Wales and Scotland where the independence referendum shook up British politics in 2014).

The Cotswolds is spread across six shires (where a shire means a county) – Oxfordshire, Gloucestershire, Worcestershire, Warwickshire, Wiltshire and Somerset. This area is perhaps England's most famous tourist region, and William Shakespeare was born and raised in Stratford-upon-Avon, a town in south Warwickshire.

Streets in the Cotswolds villages are typically lined with honey coloured stone houses and thatched cottages. A variety of small to large villages scattered through the Cotswolds offer their individual charm and uniqueness.

Chipping Norton, formerly an important wool-trading town, is on the edge of the north Cotswolds and known for its many antique shops.

Chipping Norton was ranked tenth in 2014 on the list of the most glamorous places to live by *The Times* (British daily newspaper). In this context "glamorous" means attractive, thus this list suggests the most popular places to live in the UK.

In addition, Chipping Norton is one of the current Prime Minister David Cameron's constituencies and his weekend home is at a nearby village. We sometimes visit another Cotswolds village named Great Tews (ranked second on the list mentioned above) for company socials at a country pub with accommodation called The Falkland Arms where Prime Minister David Cameron occasionally turns up with his family for drinks.

Chipping Norton is located about 100 km away from London, if travelling by train, it will take a little over one hour to reach here. Please pay us a visit whenever you visit the UK – one day excursion during your stay in London or a short break on your way to Peter Rabbit in the Lake District.

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