Proceedings of

The 6th International Conference on

Nutrition and Aging

Advanced Aging and Wellness

- From Food Supply to Dietary Habits

September 28-30, 2011
The University of Tokyo
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Edited by

International Life Sciences Institute Japan

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Organized by
International Life Sciences Institute Japan

Co-organized by
International Life Sciences Institute (ILSI)
ILSI Europe
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In collaboration with
Ministry of Agriculture, Forestry and Fisheries
Institute of Gerontology, the University of Tokyo
Japan Society of Nutrition and Food Science
The Japan Dietetic Association
Society for Applied Gerontology – Japan
Japan Society for Biomedical Gerontology
The Japanese Society of Clinical Nutrition
The Vitamin Society of Japan
National Institute of Health and Nutrition

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PREFACE

The 6th International Conference on Nutrition and Aging commemorated the 30th anniversary of the founding of ILSI Japan, and was part of a series of symposia held every four years. The first symposium was the idea of our first Chairman Tetsujiro Ohara and was held in 1991 to commemorate the 10th anniversary of ILSI Japan. That first symposium was an event held on a grand scale at Tokyo's Keio Plaza, and from that beginning has continued for 20 years. Dr. Ohara was not able to attend the entire symposium doe to his physical condition, but did make an appearance long enough to provide opening comments from his wheelchair.

It was the opinion of Dr. Alex Malaspina, the first Chairman of ILSI, that since the Japanese population leads the world in longevity, the 2nd symposium should also be held in Japan. From that start, the third, fourth and fifth symposia were also held in Japan, at four year intervals. For each symposium topics of current interest related to aging and nutrition are chosen and researchers from both Japan and abroad are assembled.

This time, the symposium was structured around the theme "Advanced Aging and Wellness - From Food Supply to Dietary Habits". As Japan continues to be a super-aging society, topics related to enabling healthy elderly years such as proper nutrition and physical activity were discussed. The program was divided into 5 sections: 1) "Topics for an Aging Society", 2) "Food Selection-What to Choose and When to Eat?", 3) "Food Culture and the Structure of Disease", 4) "The Role of Physical Activity and Nutrition", and 5) "Nutrition and the Aging and the Brain", which included international trends and provided ideas from a very broad perspective.

In addition, a satellite meeting "Nutrition and Anti-Aging - Scientific Evidence from Genomics" was held under the guidance of Professor Keiko Abe (The University of Tokyo, Graduate School) and Associate Professor Yuji Nakai to present the scientific results from ILSI Japan's Endowed Chair on Nutrigenomics. ILSI Japan committees and task forces also presented their results and related topics.

On March 11, 2011, Japan experienced a once in a millennium disaster, the Great East Japan Earthquake, which caused in a huge tsunami and extensive damage to nuclear power plants. This resulting devastation and suffering are impossible to describe. There were many reports of conditions which negatively affected the health of the elderly. Worsening environmental and health conditions appear to have the elderly in a difficult situation. Therefore, the topic "Wellness of a Super-Aging Society" was especially appropriate. I believe it is very significant that despite taking place in the shadow of such an emergency, a large number of people attended and there was much lively debate and discussion.

September 2012

Shuichi Kimura, Chairman
International Life Sciences Institute Japan
(NPO)

Chapter 1.

Topic for an Aging Society

Supply/Demand of Food, and Issues for the Future

Seiji Mitsuishi*

"What are the most important issues when we consider the future of food?" This paper reviews the basic supply and demand of grain and oilseeds both Japan and the world, and I would like to touch briefly on the outlook for the world's population prospects first as its premise. (Table 1, Figure 1)

According to data from the United Nations, the world population is currently about 7 billion people, and is expected to exceed 9 billion by the year 2050, and then reach 10 billion in 2085. Briefly, there will be an increase of 1 billion people increase in Asia and Africa by 2050 respectively, and then, another increase of 1 billion is expected in the subsequent 35 years in Africa.

This is the future outlook of the world's population drawn by the United Nations at this time. If population increases, naturally the quantity of the food will also increase. Every problem regarding food will finally connect to the population situation, and with this kept in mind, I would like to proceed with this article.

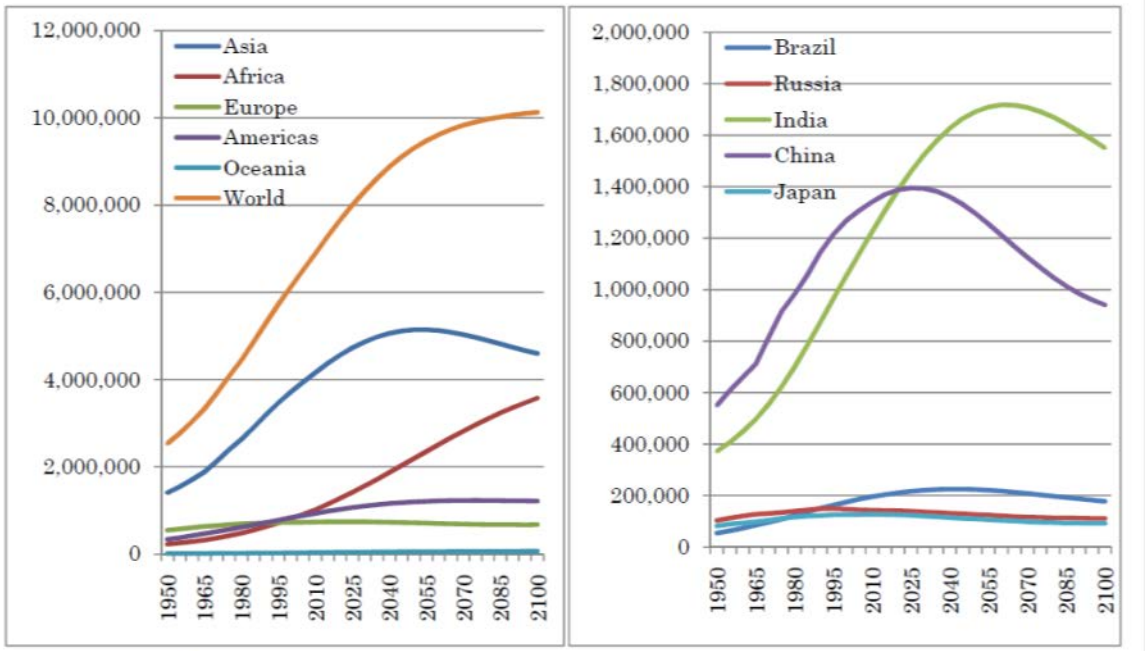
Table 1 World population prospects (1950-2100) Unit: thousand people

	Asia	Africa	Europe	America	Oceania	World	Japan	%
1950	1,403,389	229,895	547,287	338,983	12,675	2,532,229	82,199	3.25
1955	1,549,293	255,521	575,236	378,667	14,165	2,772,882	88,389	3.19
1960	1,707,682	286,729	603,854	424,376	15,773	3,038,414	92,501	3.04
1965	1,886,186	323,916	633,591	471,829	17,486	3,333,008	97,343	2.92
1970	2,134,993	368,148	655,879	517,661	19,506	3,696,187	103,710	2.81
1975	2,393,056	420,318	676,123	565,434	21,489	4,076,420	110,808	2.72
1980	2,637,586	482,803	692,869	616,780	22,970	4,453,008	115,915	2.60
1985	2,906,851	555,276	706,800	669,472	24,891	4,863,290	119,991	2.47
1990	3,199,481	635,287	720,497	724,194	26,967	5,306,426	122,251	2.30
1995	3,470,446	720,931	727,422	778,396	29,044	5,726,239	124,487	2.17
2000	3,719,044	811,101	726,777	834,718	31,130	6,122,770	125,720	2.05
2005	3,944,992	911,120	730,736	886,269	33,532	6,506,649	126,393	1.94
2010	4,164,252	1,022,234	738,199	934,611	36,593	6,895,889	126,536	1.83
2015	4,375,482	1,145,316	742,067	982,075	39,355	7,284,295	126,072	1.73
2020	4,565,520	1,278,199	744,177	1,026,576	42,056	7,656,528	124,804	1.63
2025	4,730,130	1,417,057	743,890	1,067,250	44,651	8,002,978	122,771	1.53
2030	4,867,741	1,562,047	741,233	1,103,263	47,096	8,321,380	120,218	1.44
2035	4,978,236	1,713,090	736,922	1,134,252	49,367	8,611,867	117,349	1.36
2040	5,060,964	1,869,561	731,826	1,160,215	51,475	8,874,041	114,340	1.29
2045	5,115,457	2,029,824	726,029	1,181,277	53,435	9,106,022	111,366	1.22
2050	5,142,220	2,191,599	719,257	1,197,818	55,233	9,306,127	108,549	1.17
2055	5,143,717	2,352,922	711,147	1,210,226	56,900	9,474,912	105,879	1.12
2060	5,122,743	2,512,188	702,347	1,219,506	58,405	9,615,189	103,241	1.07
2065	5,083,243	2,668,299	693,908	1,225,990	59,763	9,731,203	100,608	1.03
2070	5,029,489	2,820,005	686,745	1,229,876	60,997	9,827,112	98,126	1.00
2075	4,964,787	2,966,011	681,329	1,231,229	62,112	9,905,468	95,984	0.97
2080	4,892,292	3,105,039	677,700	1,230,402	63,106	9,968,539	94,365	0.95
2085	4,816,176	3,236,044	675,611	1,227,817	63,964	10,019,612	93,184	0.93
2090	4,740,484	3,358,296	674,657	1,223,956	64,697	10,062,090	92,345	0.92
2095	4,666,937	3,471,176	674,451	1,219,220	65,316	10,097,100	91,746	0.91
2100	4,596,224	3,574,141	674,796	1,213,945	65,819	10,124,925	91,330	0.90

Source: World Population Prostects 2010 Revision

* Professor, School of Food, Agricultural and Environmental Sciences, Miyagi University, Miyagi, Japan

Figure 1 World population prospects (1950-2100) Unit: thousand



Source : World Population Prospects 2010 Revision

Table 2 World supply/demand of grain and oilseeds (2011/12)

	Wheat	Rice	Coarse grains	Oilseeds	Total
Carryover	193,339	96,293	162,902	79,970	532,504
Production	678,115	458,377	1,131,195	452,980	2,720,667
Demand	676,860	456,018	1,144,139	460,390	2,737,407
Ending Stocks	194,594	98,652	149,958	72,560	515,764
Stock-to-use Ratio(%)	28.7	21.6	13.1	15.8	18.8
Change	1,255	2,359	−12,944	−7,410	−16,740

Source:USDA (Sep. 2011). Note: Rice is milled basis.
Unit: Thousand MT

Supply/Demand of World Grains/Oilseeds

The basic data on the supply and demand situation of wheat, rice, coarse grains, and oilseeds in the 2011/12 year released by the USDA or United States Department of Agriculture announced on September 11, 2011 is shown in the Table 2. This served as a very important basic data when we examined the world grain supply and demand situation, and the USDA issues it around the 10th of every month. There were four important categories in grain and oilseeds: “wheat” which is the most important food grain in the European and American societies, “rice” as the most important food in Asia, “coarse grains” which included corn as the most important ingredient for feed and for industrial use, and finally “oilseeds” which included soybean for crushing, for other products, and livestock feed.

In order to grasp the summary of the supply and demand situation, the crux was the overall amount of production and demand quantity, and not the details of the individual items. Table 2 shows the contents, and it was important to understand that the total amount of production and the total demand were approximately 2.7 billion tons respectively. The stock-to-use ratio was 18.8% (crop year of the USDA ends in August) and approximately 17 million tons decreased compared to the previous year.

The major reason for this change was the decreasing number of ending stock of coarse grains, mainly corn. In the case of the 2011/12 year, a decrease in corn stock really affected the whole balance since corn accounted for about 76% of total coarse grains production. But it is necessary to understand that there is a strong demand increase at a rate exceeding that of production increase although corn production has increased steadily in the long term. Bioethanol is the strongest demand factor within the U.S., and increasing feed demand in many emerging countries is another demand pushing factor outside the U.S.

It is worth noting that the biggest change I noticed during the past 15 years in the entire grain and oilseeds was the changing "position" of oilseeds particularly in soybean and its products in the global market. Figure 2 shows this change that happened in the world trade after the 2000s. Historically, the trade quantity of soybean and its products including soybean meal and soybean oil were less than those of wheat and coarse grains which were used as staple food and major feed ingredients respectively. However, it has clearly changed since the beginning of the 2000s, and it is anticipated that this trend will continue semi permanently in future.

Figure 2 Global trade: wheat, coarse grains and soybeans and soybean products (Source: USDA)

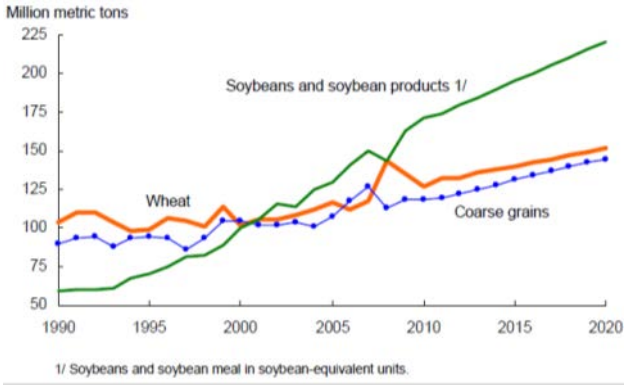


Figure 3 and Figure 4 explain why this trend occurred. Figure 3 shows the global soybean export since 1990, and the prospect until 2020, and Figure 4 expresses world soybean import in the same timeframe. The export of soybeans was the highest in the U.S. traditionally, but, from the mid-1990s, exportations by Southern Hemisphere countries such as Brazil and Argentina grew export rapidly.

Figure 3 Global soybean export (Source: USDA)

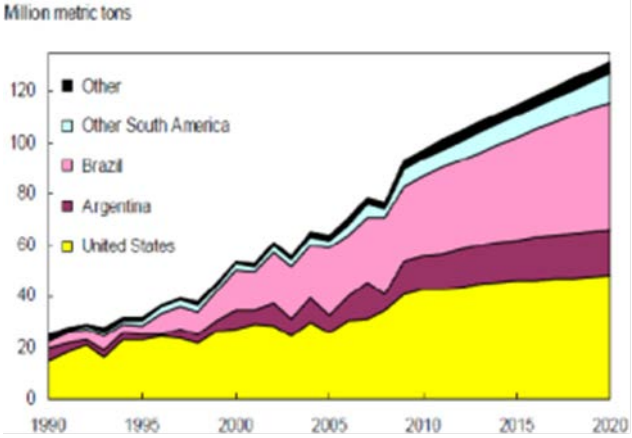
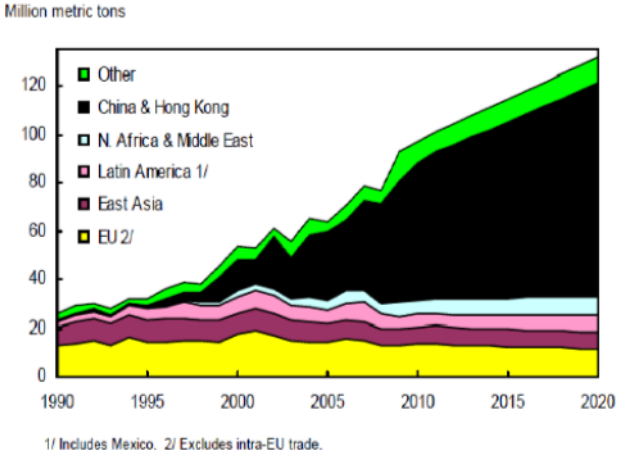


Figure 4 Global soybean import (Source: USDA)



On the other hand, it was China to increase soybean import dramatically in the form corresponding to this rapid increase of production since the same mid-1990s. The soybean import by China was 37.8 million tons in the 2007/08 year, but 56.5 million tons is anticipated in the 2011/12 year. In the 2011/12 year, the export of world soybean amount is estimated to be 98.3 million tons, but China will import about half or more.

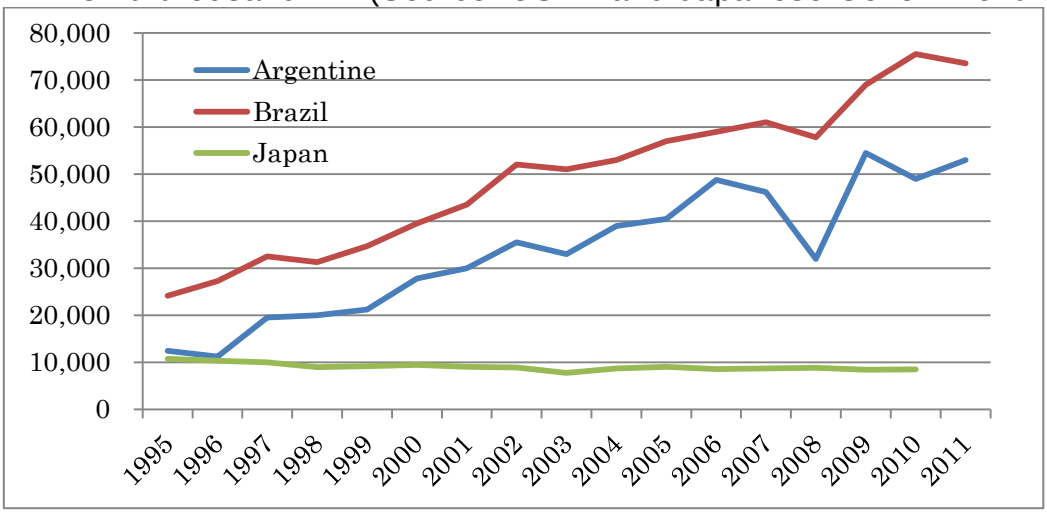
This is attributed to the recent economic growth and improvement in the standard of living in China, and it is the greatest and inevitable cause that the eating habits of the nation has changed greatly by the standard of living having improved. The oilseeds including soybean were the raw materials for the vegetable oil, and, after crushing oilseeds, the meals became an important feed for domestic livestock. A strong domestic demand for both soybeans for crushing and for livestock feeding coupled with the changing eating habits in China all contributed to the drastic increase in soybean import. Interestingly, the importation of all oilseeds in China for the 2011/12 year was 57.5 million tons. Therefore, the ratio of soybean among the whole oilseeds import of China is remarkably high at 98%, which seems to be part of strategic focus in China.

On the other hand, it was the South American countries Brazil and Argentina that responded to the increase of such a sudden demand. Figure 5 shows the changes in the soybean production of these two countries about the same period since 1990s.

At the time of 1995, the soybean production in Brazil was approximately 24 million tons while that of Argentina was approximately 12 million tons in Argentina. However, the respective estimates for this year are 73.5 million tons and 53 million tons, and combined current production will easily exceed 100 million tons for these two countries. There are several background reasons why these two countries extended soybean production rapidly by exploring uncultivated large land, but at the same time, we cannot miss the huge impact of biotechnology or specifically the expansion of genetically modified organisms.

Northern and Central Argentina as well as Southern Brazil are the traditional soybean producing areas. When the commercial production of genetically-modified crops was started in 1996, the introduction of genetically-modified crops was carried out immediately in Argentina. In contrast, genetically-modified crops were still illegal in "then" Brazil, but it was next to Argentina geographically and the climate was very similar, and, at the straight production center of the near southern part. As a result, the seeds of genetically modified soybeans were brought in on the basis of many individuals from Argentina, and the planting expanded quite rapidly. During the time of 2002 through 2003, in the southern state of Rio Grande do Sul, it was reported to be roughly 70% of all soybeans planted were genetically-modified varieties

Figure 5 Production of soybean (Argentina and Brazil) and Rice (Japan)
Unit: thousand MT (Source: USDA and Japanese Government Data)



Not only did this situation considerably progress afterwards, but also, the problem of the license charges by the use of genetically-modified seeds through illegal use surfaced as a big problem on the side of development companies. Furthermore, at the international level, even the Brazilian soybean importing countries started to show their interest because the argument about import approval and the procedure for importing genetically modified crops were still in progress in these countries.

On the other hand, the Brazilian soybean production ground in the central and western areas or Centro-Oeste was different from the southern part. In this area, soybean production increased by the progress of development. In the state of Mato Grosso or Goias, huge soybean fields were newly born one after the other.

By the establishment of the official Biosafety Act at in 2005, the planting, distribution, and selling of genetically-modified soybean were approved in Brazil and that lead to the production of soybean in that country.

In addition, the data of the amount of “rice” harvest of Japan, in the same period can be superimposed as shown in Figure 5. Because the conditions of each country are different and the crop itself is also different, this comparison might not mean much in the sense of supply and demand or any agronomical sense, but it is quite interesting when we look at it from the viewpoint of the kind of trends major crops like the soybean of South American two countries and rice in Japan followed during past 15 years.

The potential amount of rice production in Japan used to be between 16 million tons and 20 million tons, but it has now dropped to 8.5 million tons. Rice production in Japan will no more place even within the first ten of the world production ranking. The world's largest rice producing country was China, and the volume was approximately 16 times that of Japan, and production prospect for the year 2011/12 is 139 million tons, which is far superior.

In terms of rice trade, the world's largest rice exporting country was Thailand with 8 million tons. Thailand’s rice production was 20 million tons, and they exported approximately 40% of the entire production. The world trade amount of rice was a little over 30 million tons, but Thailand and Vietnam with 6.4 million tons of export, accounted for almost half the global exportation.

The Situation of Japan

So, how much grains does Japan import annually? Table 3 is a compilation of imported grains and oilseeds to Japan after 2007. There are some annual fluctuations, but it is clear that Japan imports around 30 million tons every year. In the year 2011/12, Japan is expected to import 5.8 million tons of wheat, 0.7 million tons of rice, 19.1 million tons of coarse grains, and 5.92 million tons of oilseeds.

Out of the coarse grains, approximately 16 million tons is corn, while approximately 3.4 million tons out of the oilseeds is soybean. As for corn, the feed use is approximately 12 million tons and the remainder is food use such as corn starch and other industrial use. This clearly shows the fact that Japan needs 1 million tons of corn every month to respond to the domestic livestock demand. Generally, feed which comes in the form of a mixture of various cereals in the most suitable ratio called the “compound feed” is used in the livestock industry of Japan. In this compound feed, about half of the ingredients is corn. Therefore, the importation of stable corn is indispensable for the stock-raising industry in Japan.

Because such a mass transportation of grains has become "a commonplace" today, many people may not notice it, but it is carried out by the constant effort of companies and organizations such as many grain producers, a carrier, and safekeeping suppliers engaged in it. This global social system has been functioning as "the invisible infrastructure" for Japan which is not able to produce sufficient grain to meet its domestic demand.

Table 3 Japanese grain import (unit: thousand MT)

	2007/08	2008/09	2009/10	2010/11	2011/12
Wheat	5,701	5,156	5,502	5,869	5,800
Rice	533	750	649	700	700
Coarse Grains	19,210	19,611	19,198	18,245	19,135
Oilseeds	6,520	5,740	5,910	5,710	5,920
Total	31,964	31,257	31,259	30,524	31,555

Source：USDA（Sep. 2011）. Note: Rice is milled basis.

Table 4 Japanese import of GM crops (Estimates, unit: 10 thousand MT)

Coarse Grains	Corn	1,610	＝1,610X89%X88%≒	1,260
	Barley	130		0
	Sorghum	160		0
	Rye	8		0
	Oats	6		0
	s.ttl	1,914		1,300
Oilseeds	Soybean	340	= (340−80)X71%x94%≒	174
	Canola/others	225/27	= 225X92%X94%≒	195
	s.ttl	592		369
	G.ttl			1,629

Note: Author's calculation based on USDA and ISAAA data.
1st percentage is a ration of import from the U.S., and second percentage is planted percentage of GM varietirs in the U.S.
Soybean figures are ajusted by deducted IP handling volume as 0.8MT.

By the way, how many tons of genetically-modified crops are included in the approximately imported 30 million tons a year? In fact, the accurate answer to this question does not exist. As a result of having evaluated safety for many years, the U.S. Government upon which Japan depends for most of the imported grain and oilseeds does not distinguish between the genetic modification crops and the conventional crops substantially.

Therefore, the only way one can precisely determine the quantity of imported genetically-modified crops is to perform a rational estimate. The calculations are shown in Table 4. As illustrations, 16.1 million tons of corn was imported. In the case of corn and 89% of the import comes from the U.S. The planting ratio of genetically-modified corn in the U.S. is 88%. Based on this assumption, a figure of around 12.6 million tons may be used for the calculation. The calculation is basically similar for soybean and the canola. In addition, even though the number of 16.29 million tons is displayed in Table 4, it will be of no use trying to figure out small details. Rather, it would be better to comprehend it with rounded numbers when a minimum of about 16 million to 17 million tons on the whole is imported.

Understanding the Genetically-Modified Crops

The situation that "nobody still knows" continues the general sentiment around genetically-modified crops in current Japan. While our everyday life was established by importing large amounts of grain and oilseeds constantly, not much deliberations from the front has been done unfortunately.

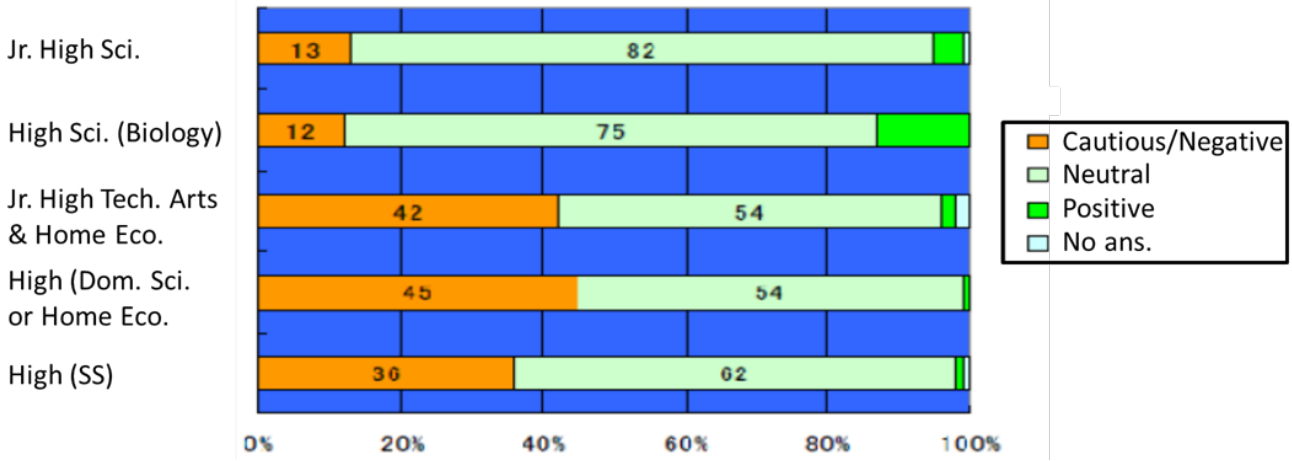
While Japan continued to stagnated, Table 5 shows one end of the reality of the situation the world finds itself. By considering the case of soybean as of 2008, herbicide-tolerance was the only event commercialized. However, it is anticipated that 17 new events would have already been in the final development stage or possibly in the market by 2015. There already are 10 new events in the final stage of the development. A figure 15 is shown even with rice by 2015.

Table 5 Event in commercial GM crops and in pipelines worldwide, by trait & by region

Crop	Commercial in 2008	Commercial pipeline	Regulatory pipeline	Advanced development	Total by 2015
soybeans	1	2	4	10	17
Maize	9	3	5	7	24
Rapeseed	4	0	1	5	10
Cotton	12	1	5	9	27
Rice	0	1	4	10	15
Potatoes	0	0	3	5	8
Other crops	7	0	2	14	23
All crops	33	7	24	60	124
Developer country	**Commercial in 2008**	**Commercial pipeline**	**Regulatory pipeline**	**Advanced development**	**Total by 2015**
USA & Europe	24	7	10	26	67
Asia	9	0	11	34	54
Latin America	0	0	2	1	3
Total	33	7	23	61	124

Source: European Commission–JRC Science and Technological Reports, "The Global Pipeline of new GM crops", 2009.

Figure 6 Attitude/consciousness of 4,080 teachers in classroom (%)



Furthermore, when we consider the breakdown of 124 in total according to an area, not only from Europe and the U.S. but also Asian countries would commercialize 54 new products. A lot of attention needs to be focused on this.

At the private enterprise level, it is necessary for each country wrestling in such research and development at a national level, to understand the reality that there are many wells to secure growth and the competitiveness of the future regardless of the distinction between developing and the developed countries.

In this sense, the support, attitude and careful attention of Europe should remain calm, while in other research and development for the future competitiveness of the country are continuing.

One interesting aspect of Japanese situation is shown in Figure 6. This explains a kind of stance and attitude in mind, when teachers taught genetically-modified crops and food in their classroom. The answers came from 4,080 junior high school and high school teachers. The survey was conducted by the Japanese Government's Cabinet Office in 2008 as part of a research in "an attitude survey about the spread of re-

search and development by the genetically-modified technique".

In the case of natural science teachers, most took the neutral viewpoint, and the extreme agreement, cautious and negative attitudes were the minority to the last. However, it was the home economics teachers that scored higher than 40% in carefulness and passivity. Three years have already passed since this research was conducted. Junior high student who learned with this stance have now become high school students, while the high school student are now likely to be to university students. The situation has not possibly changed greatly from the point of this research.

For the future of this technology, along with efforts to develop cutting –edge research from the front, a major challenge may be how to work cooperatively with teachers in fields such as food education, agriculture, home economics, and nutrition and so on. Continuous efforts of transmission about correct scientific knowledge to teachers and dieticians related to such a field will be necessary, too.

More interesting data is shown in Table 6. This settled the attitude and consciousness of 197 local government staff in the same investigation. The staff working in the food safety, agriculture and hygiene section, generally might have scientific knowledge or a scientific background tended to take on consciousness, "significantly safe, or relatively safe" to the human being for consumption of genetically modified crops and food. On the other hand, the staff working in the consumption and life related sections where there were many opportunities to cope with consumers directly tended to take on attitude, “relatively risky”.

From the viewpoint of the general public, it is the government itself has a different opinion about the safety of genetically-modified crops and food. Even if this is just “consciousness", and there are official government policy about genetically-modified crops, in the organization, it will be necessary for such situation to improve immediately because the total performance of organization is always influenced by its consciousness, attitudes, and behavior of the members.

Five Elements for the Future

There are at least five important elements to recognize before we consider future of food, and these elements may be considered as “the uncertainties” in other words. The world’s population will surpass 10 billion people in 2085 if the projection of the United Nations is right. If the increase and decrease of the population draws a constant bell curve, as the United Nations expects, the peak of the population of the human is more likely to come in the first half in the 22nd century. In any case, during the next 100 years or so, what uncertainties will we face with this premise of continuing increase of population?

Table 6 Attitude/Consciousness of 197 local government staff (%)

	Very safe	Relatively safe	Relatively risky	Very Risky
Total	10.3	48.7	33.8	1.5
Food safety & Hygiene Dept.	15.2	68.2	12.1	0.0
Ag related Dept.	9.9	57.7	25.4	2.8
Consumer & Life related Dept.	5.6	14.8	72.2	1.9

Source: Japanese Cabinet Office Report, 2008.

What first comes into mind is land, water, a climate change, energy and technology. Various movements have been already announced about land and water. From the viewpoint of the supply and demand of food in the short-term, it is South America that has most has vitality on earth now. But in the long-term, the land with the highest potential value of output will be the Africa. How we deal with this will be the most important issues from now on. Water will be a huge issue, too. Most people in the present day Japan, including myself have never really experienced water scarcity problems. However, this is not the issue of others. We do not need to argument about the virtual water. To put it simply, those who live in a country with huge amounts of grain imports will actively and sincerely consider the responsibility of it. It may not be exciting as the climate change like before, but it is still a big element of uncertainty. We can never wipe the risk in the first place since the future estimate itself will vary widely.

Because of the great earthquake disaster in the eastern part of Japan and the problem of the Fukushima Nuclear Power Plant, the attitude towards the energy issues may change, but there are still many uncertainties. Enormous time, labor, and money will be required even to clean up just one nuclear power plant. Two actions are required: one is quick response, and the other is careful planning and executing it for a long time (be sure someone will) and support must be made. Both are required to proceed under international agreements and with patience together in parallel.

The issue of food may break out in a similar "sumo ring" sometime soon, too. To that end, we need to seize an opportunity and should carry out an action of international problem's solution. If we fall into a viewpoint of the domestic conclusion only, we would miss bigger issues of the outside world.

Conclusion

When I look at world's average of crop production per hectare, the yield of wheat is still currently around 3 tons. Corn is 5 tons, rice is 4.3 tons, and soybean is 2.5 tons. There are many countries that have their average crop yields under the world average. Science is not a panacea, but our civilization and real life certainly depends on science and science-based technology. If it is the future course that population growth is not avoided, and it becomes a reality, we cannot but choose a direction of activating potentiality of nature and the crops, in a form that does not become too arrogant.

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Chapter 2.

Food Selection
- What to Choose and When to Eat?

Health Promotion and Food Choice

Yoshiko Ishimi*

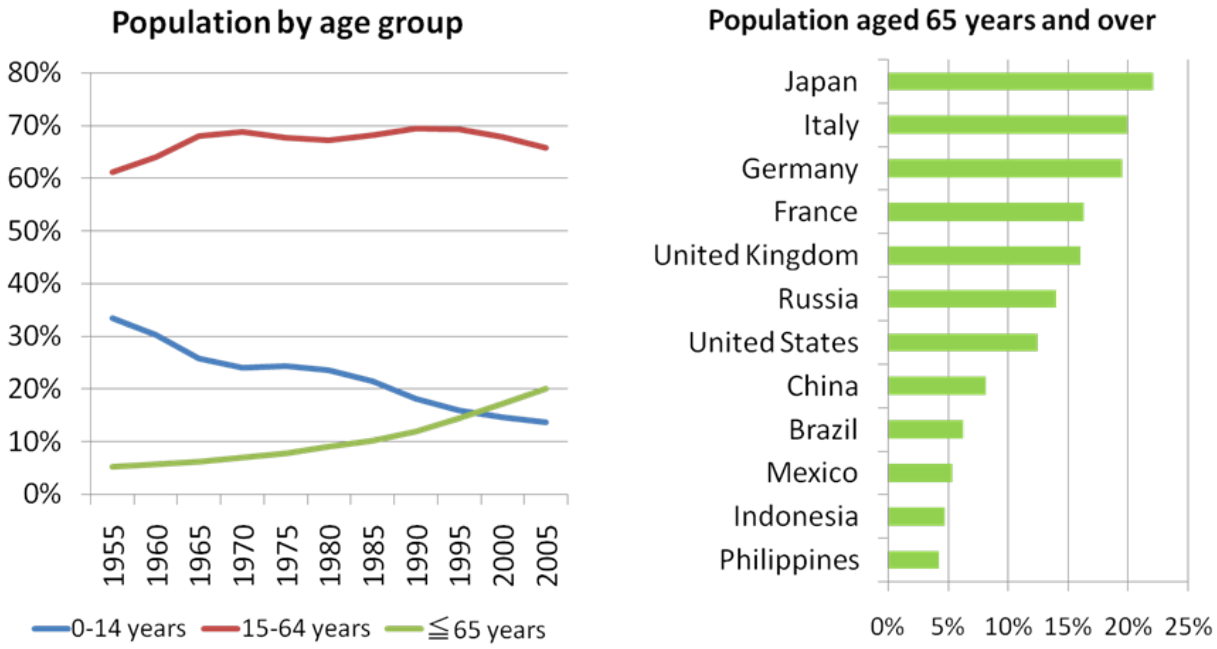
Age structure of Japanese Population

Elderly people now account for approximately 23% of the total population of Japan, which is becoming a super-aged society (Fig1) [1]. Needless to say, the proper diet and physical exercise from a young age are important factors for preventing the occurrence of lifestyle-related diseases, and ensuring a healthy old age.

National Health and Nutrition Survey Japan

According to the results of National Health and Nutrition Survey Japan (NNS-J), the ratio of overweight in middle aged males was increasing; on the other hand the ratio of underweight of females was increasing in Japan (Figure 2).From the results of NNS-J 1946-2000, the intake of rice, grains, and potatoes were decreasing, while the intake of meat, egg, and milk were increasing (Figure 3). In addition, although it has been on a downward trend in recent years, the fiscal 2009 survey showed that the intake of salt per day remained high at a total average of 10.7 grams for people aged 20 years or over [2]. Unfortunately, the number of people who do not eat breakfast was high in 20 – 49 years old males and females (Figure 4) [2].

Figure 1 Age structure of Japanese population



* PhD, Department of Food Function and Labeling, National Institute of Health and Nutrition

Figure 2 Percentage of overweight (BMI ≥25) by age and sex (1982-2002 National Nutrition Survey Japan)

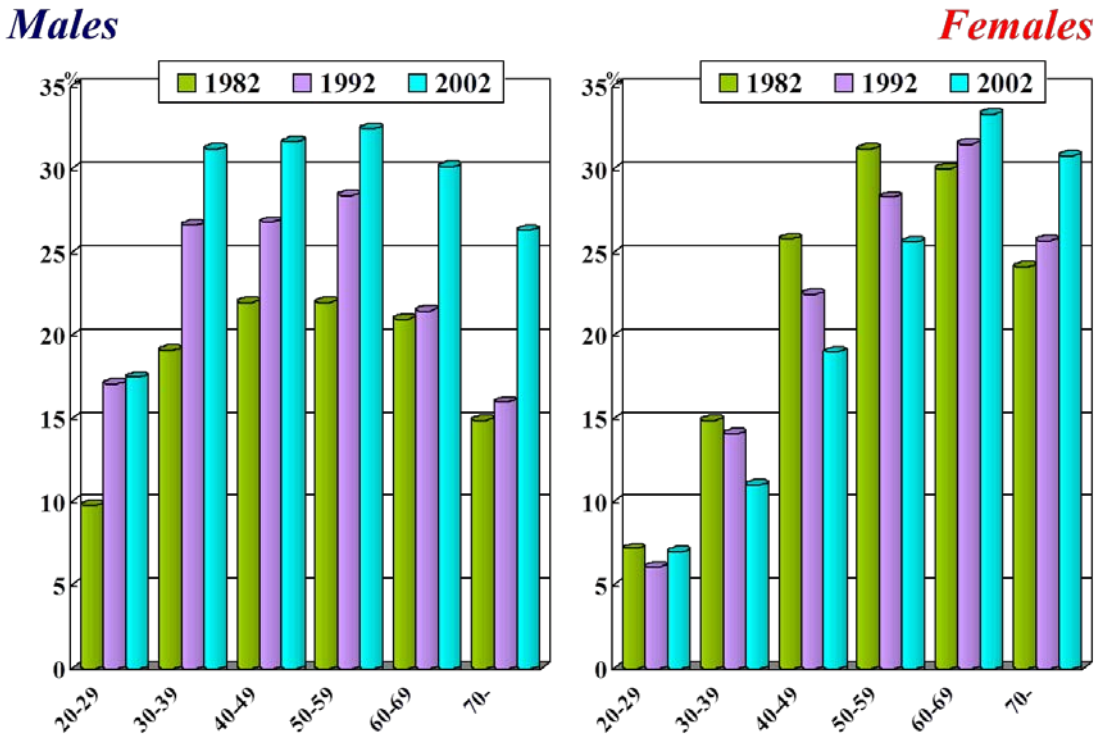


Figure 3 Changes in food intake per capita per day (1946-2000 National Nutrition Survey Japan)

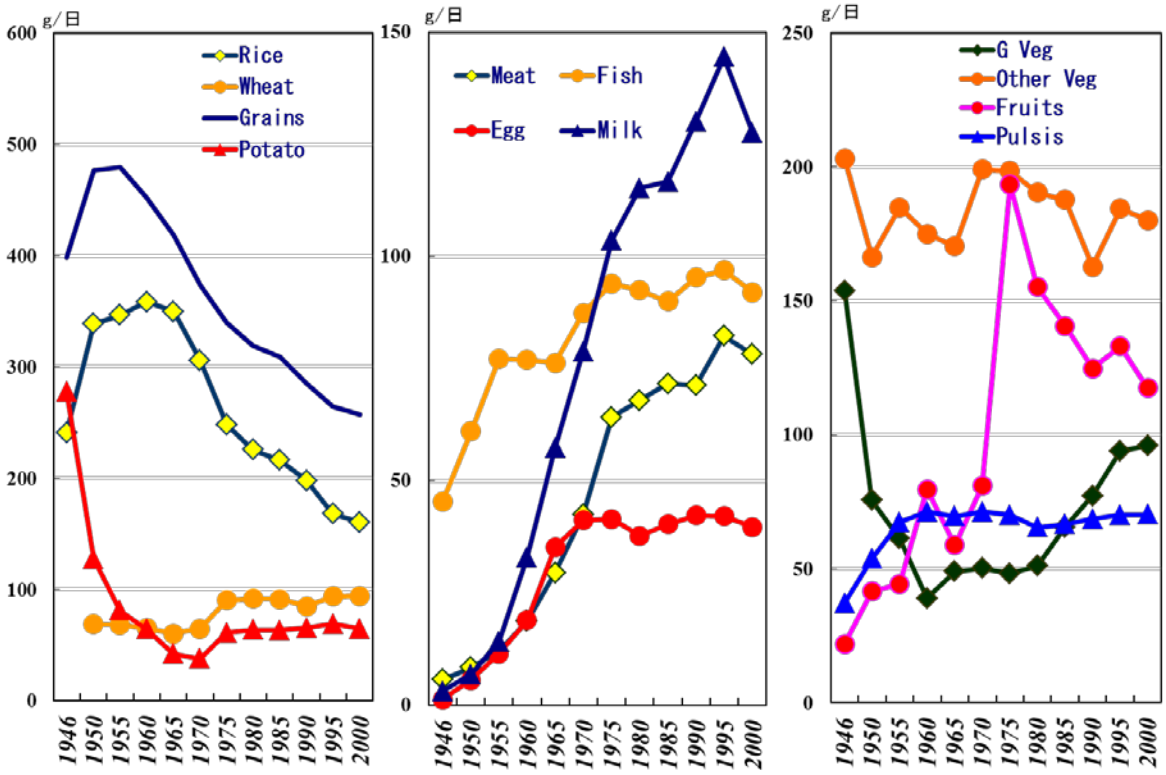
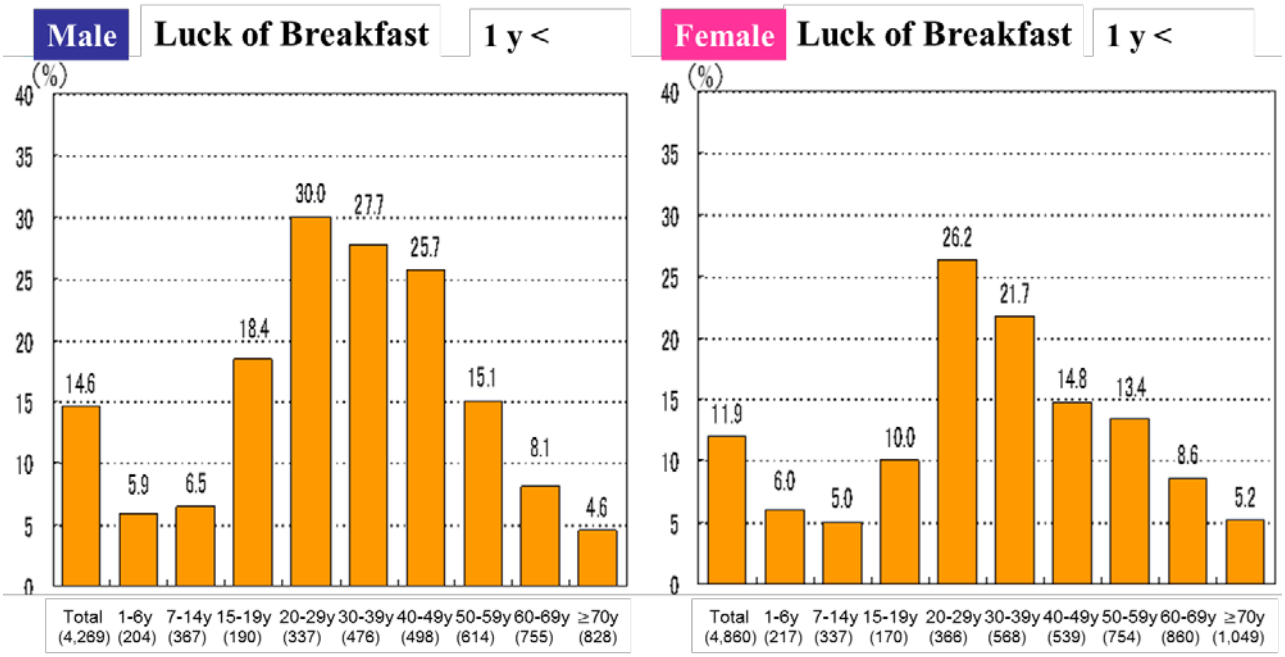


Figure 4 The number of people who do not eat breakfast (2008 National Health and Nutrition Survey Japan)



Health Policies of Japanese Government

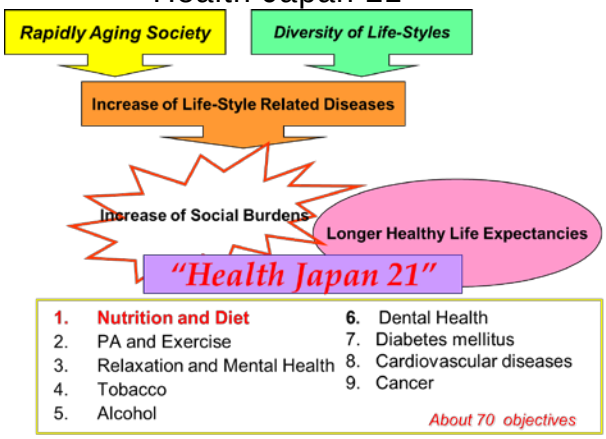
From these backgrounds, Japanese government has been developing the policies for health promotion (Figure 5). As one of the health policies of the government, specific targets for nutrition and diet, physical exercise, and so on are indicated in the "Health Japan 21" campaign (Figure 6) [3].

Figure 5 Guidelines for Health promotion in Japan

- ✔ Health Japan 21 :2000~2012
- ✔ Dietary Guidelines : (1985) 2000 ~
- ✔ Dietary Guide Spinning Top : 2005~
- ✔ Dietary Reference Intakes for Japanese, 2010
- ✔ Food and Nutrition Education (Shokuiku):2005~
- ✔ Exercise and Physical Activity Reference for Health Promotion 2006
- ✔ Food Labeling System : 1995~
- ✔ Specific Health Checkups and Health Guidance : 2008~

Regarding nutrition and diet, prefectures and municipalities are compiling and implementing health promotion plans that set targets for proper food intake and behavioral changes and environment building for this purpose. In addition, in 2000 the Ministry of Health and Welfare, the Ministry of Agriculture, Forestry, and Fisheries, and the Ministry of Education, Science and Culture jointly compiled "Dietary Guidelines" for the realization of a high-quality dietary lifestyle emphasizing enhancement of the quality of life [4]. Furthermore, the ministries have issued the new Japanese "Food Guide" in 2005 (Figure 7), which informs the nation what should be eaten and how much in an easy-to-understand manner [5].

Figure 6 Background in development of "Health Japan 21"



On the level of nutrients, meanwhile, the Ministry of Health, Labour, and Welfare has compiled the "Dietary Reference Intakes for Japanese (2010)", which are standards for energy and nutrient intake aimed at maintaining and promoting health and preventing lifestyle-related diseases [6]. Furthermore, regarding physical exercise, the Ministry of Health, Labour, and Welfare compiled the "Exercise and Physical Activity Reference for Health Promotion 2006" with the aim of preventing lifestyle-related diseases [7]. Japanese government also developed "Basic Low on Food and Nutrition Education (Shokuiku)" in 2005 in order to acquire the knowledge about food as well as the ability to make appropriate food choices through various experience related to foods, in order to develop people in the ability to practice a healthy diet [8]. In addition, the Food with Health Claims system has been established as a system for regulation of food labeling to correctly inform people about nutrition and health information of foods [9].

Figure 7 New Japanese Food Guide (2005)

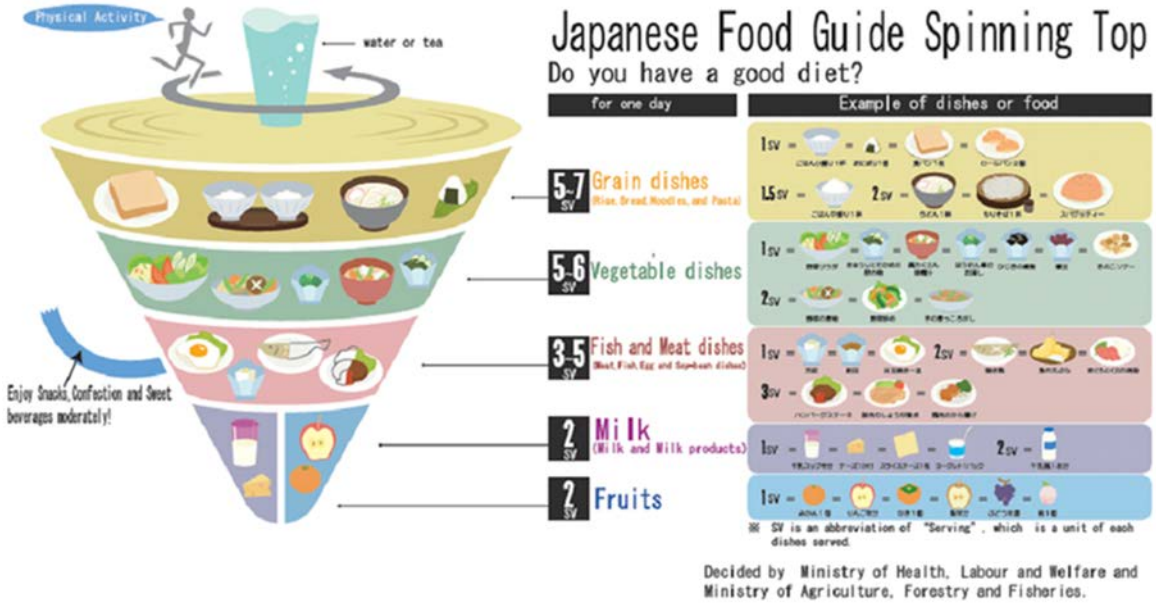


Figure 8 Relationship between saturated fatty acid intake and risk of cardiovascular disease (Ref. 11)

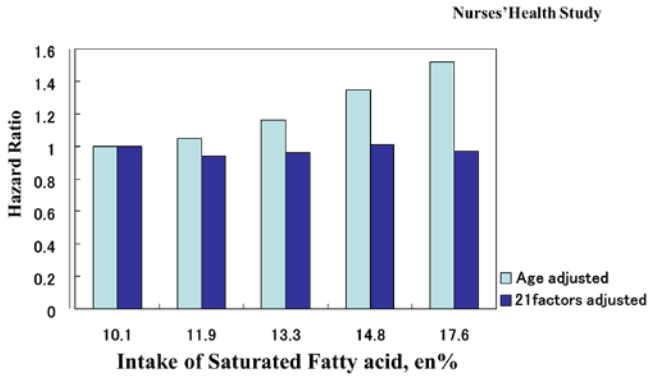
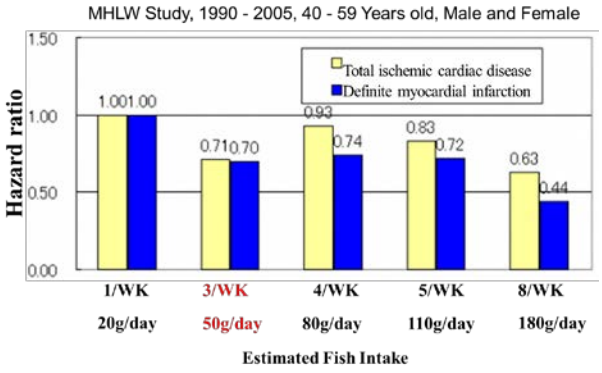


Figure 9 Relationship between fish intake and risk of cardiovascular disease (Ref. 12)



Diet and Prevention of lifestyle-related diseases

It is on the basis of these health and nutrition policies of the government that we choice our foods and conduct our lives according to our individual intentions. On the other hand, the ratio of death due to life-style-related diseases, such as hypertension, cardiovascular disease, cerebrovascular disease, diabetes, and cancer, accounts for 60% of the total number of deaths. It has been reported that the relationship between saturated fatty acid intake and risk of stroke and cardiovascular diseases (Figure 8) [10, 11]. While, as the epidemiological studies in Japan, intake of fish (3 days/week) (Figure 9), fruits and vegetable or soy foods (5 days/week) (Figure10) correlated with lower hazard ratio in cardiovascular diseases and stroke [12-14]. On the other hand, there has been reported that the relationship between higher salt intakes and risk of stroke [15]. Aside from these issues, the study also has been done to clarify the relationship between dietary pattern and prevention of lifestyle-related diseases [16].

Figure 10 Relationship between soy intake and risk of stroke and cardiovascular disease (Ref. 14)

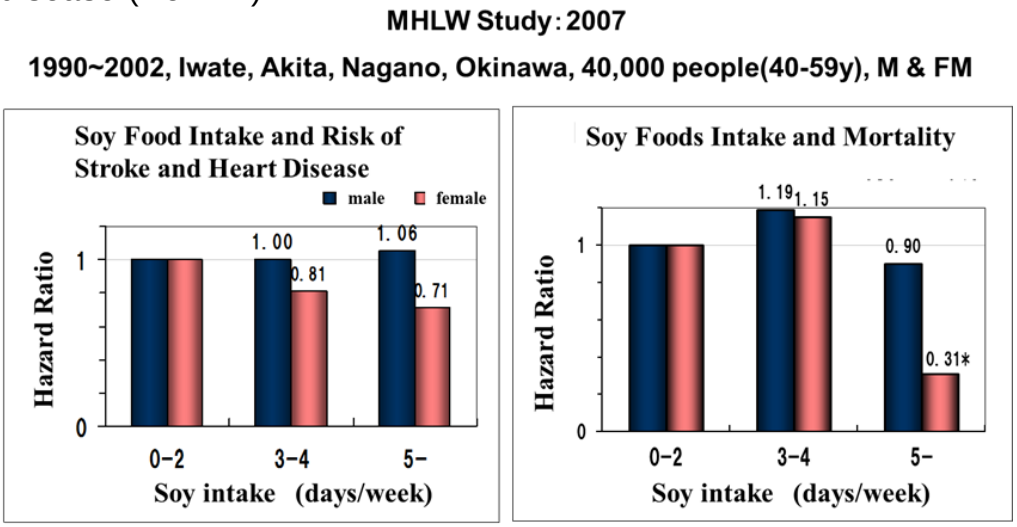
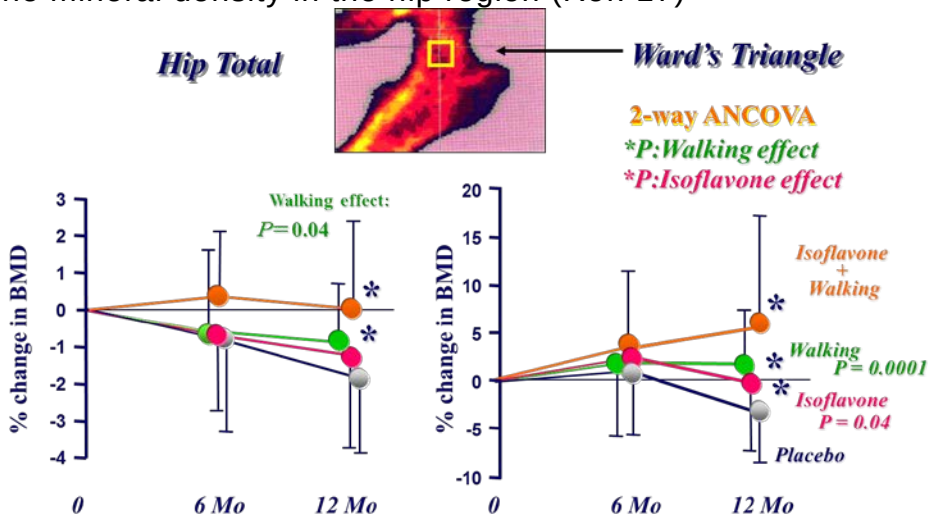


Figure 11 Cooperative effects of soybean isoflavones and exercise on bone metabolism in postmenopausal Japanese women: time course of % change in bone mineral density in the hip region (Ref. 17)



Prevention of Osteoporosis

As usual, there was a shortage in the intake of calcium, especially among adult men and women. It has been pointed out that elderly people in particular require more vitamins that play an important role in bone metabolism, such as vitamin D and K. In view of the fact that the occurrence of osteoporosis is increasing year by year, and fractures are the third highest cause for becoming bedridden, the maintenance of healthy bones is an important factor in extending a person's healthy lifespan. Therefore, we examined the cooperative effects of exercise and soy isoflavones which show the week estrogenic effects on bone metabolism in postmenopausal Japanese women (Figure 11). As the results, combined intervention of isoflavone intake and walking exercise 3 times/week for 1 year showed a trend for a greater effect on BMD at total hip and Ward's triangle regions than either alone [17].

Conclusions

As conclusions, it can be said that the ideal diet for Japanese is an intake of grains as the staple diet, fish at least three times a week, soybean products five days a week, and fruit and vegetables every day (280 g and 350 g/day, respectively), together with a moderate intake of salt and plenty of dairy products (Figure 12). This almost entirely conforms to the items cited in the "Global Strategy on Diet, Physical Activity and Health" proposed by the World Health Organization in 2004 (substituting fish for nuts) (Figure 13).

In the Japanese diet, the excessive intake of salt is a problem, but since this is closely related to such factors as Japanese food culture, including soy sauce and miso, and climate, it would be difficult to find a solution immediately. From now on, though, it is hoped that further health and nutrition policies will be developed so as to reduce the intake of salt in an efficient manner. It is also necessary to consider the impact of food culture on the food choice.

Figure 13 WHO Global strategy on diet, physical activity and health

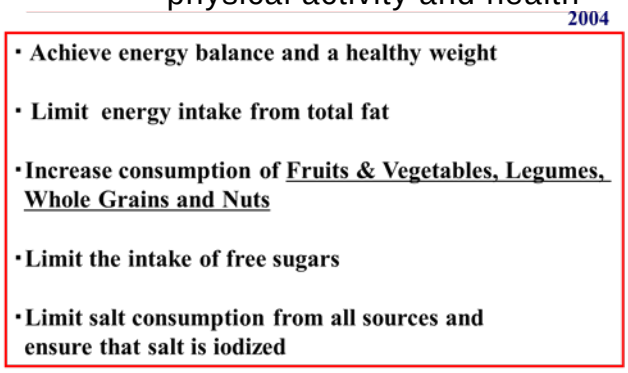


Figure 12 Food choice for health promotion

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Chrononutrition

Hiroaki Oda*

Introduction

Well-regulated eating habits are said to be important for health in both the East and the West. The importance of breakfast is emphasized in ancient Japanese books [1, 2]. We seem to have recognized the importance of the timing of meals empirically. It is generally understood that people who work at night suffer from coronary disease and obesity more frequently [3]. Furthermore, there is a relationship between shift work and cancer [4]. Nowadays, very active people in the modern society tend to have erratic schedules. Many cannot lead well-regulated lives due to their jobs, making it difficult to fix such lifestyles. However, the vast majority of these people are unaware that irregular eating habits are a major factor leading to health problems. People have inadequate knowledge of the importance of well-regulated eating habits because the mechanisms leading to the effects of the timing of meals on health are unclear. Therefore, clarifying these mechanisms may lead to the development of foods and drugs that normalize body clock.

Why is chrononutrition receiving attention now?

According to the National Health and Nutrition Survey, Japan, 2007, one-sixth to one-fifth of the Japanese are suspected to have diabetes [5]. This issue is becoming more common worldwide. Obesity is particularly increasing in men. However, caloric intake is decreasing slightly in Japan. Although it is thought that fat intake and decreasing physical activity are contributing to this problem, many people are health conscious and do exercise regularly. Then, what factor(s) are causing this problem? Meals are important. People generally think of what they should eat when they hear the word “meal.” However, it is better to think about the way to eat (“Meal Style”).

Nutritional sciences have traditionally prioritized what we eat. The “5 W’s and 1 H (5W1H) of meals” should be considered (i.e., what, when, where, who, why, and how); in other words, the way meals are eaten should be considered to better understand nutrition. Furukawa states that wealth and information are the factors controlling mean lifespan [6] and points out that slightly improving environment further extends mean lifespan. Increasing wealth makes desired foodstuffs more available. Therefore, information regarding ways to eat may be the key to increasing mean and healthy lifespans in the future.

Among the 5W1H of meals, why one eats is excluded because eating itself is the meaning and nature of life. Considering the second law of thermodynamics, metabolic turnover and dynamic equilibrium indicate that the definition of life is “eating and metabolism” in a sense [7]. This importantly reflects the fundamental significance of meals.

The most commonly performed molecular biological analyses involve the relationship between time and life. A field called “chronobiology,” which has been widely recognized as one of the basic phenomena of

* Associate Professor, Graduate School of Bioagricultural Sciences, Nagoya University

life for a long time, includes the clinically serious subject of sleep disorders.In addition to sleep, many biochemical phenomena involving rhythms were examined extensively in the 1980s. In 1971, an abnormal rhythmicity in Drosophila mutants was discovered [8], indicating that a biological clock is coded in its genes. In 1984, the clock gene of Drosophila, called Period, was discovered [9]. However, this was not a breakthrough, and further research on clock genes was performed. Meanwhile, the Japanese Society for Chronobiology was established in 1995. Mouse Period and Clock genes were cloned in 1997 [10]. A major breakthrough was the discovery of the negative regulatory feedback for transcription via the binding of CLOCK/BMAL1 to E-box, which forms the basis of biological clocks [11]. Since then, research in chronobiology has boomed. However, it was established only approximately 10 years ago and is a relatively immature discipline. We began to study DBP (albumin D-element binding protein), a transcription factor involved in circadian rhythms, as a part of a study on hepatocyte differentiation. This represented a chance for us to launch chronobiology. Just after the start of the study, we became aware of nutritional importance and subsequently progressed towards an experiment aiming to regulate meal timing, which is discussed below. In 2005, the first instance of the term "chrononutrition" appeared in a nutrition textbook edited by us [12]. The world's first book on the subject, entitled "Chrononutrition," was published in 2009 [13]. Chrononutrition, a relatively new field of nutritional sciences, is gaining recognition because it is regarded as the key that will help in understanding why there is an increase in the number of patients with obesity or metabolic syndromes in spite of a decrease in the energy intake.

Intrinsic circadian clocks

The human body has a diurnal rhythm (a phenomenon with daily periodicity) [14]. Children who sleep well are said to grow up strong, maybe because growth hormone is secreted at night. On the other hand, there are periods called danger times in which sudden death occur frequently, such as morning[14]. Physiological phenomena leading to myocardial and cerebral infarction often occur in the morning. Diurnal rhythms are observed not only with respect to when diseases occur (e.g., stomach ulcers worsen early in the morning), but also when death occurs, which varies depending on the cause of death. The Olympic finals are not held in the morning because that is not when performance is increased both psychologically and physically. Most of these time periods are controlled by intrinsic clocks. Human body is regulated by an internal clock more than we expected.

An internal clock with an approximately 24-hour cycle is called a circadian clock that is responsible for the circadian rhythm, which is different from the passive diurnal rhythm. Organisms are thought to need a circadian clock because of the predictability and functional division of labor. Predictability is essential for obtaining food and escaping from predators. Many mammals are still nocturnal because those that coexisted with dinosaurs were active at night i.e., when the dinosaurs rested. Some mammals are believed to have started becoming active in the daytime to obtain food more easily. Functional division of labor enables temporal division of labor for many complicated cell functions. In general, cell growth occurs at night, while differentiation functions required for activity occurs in the daytime. Many biochemical metabolic pathways overlap. Metabolism cannot occur smoothly unless temporal division of labor occurs. As gluconeogenesis and glycolysis systems cannot work simultaneously, temporal functional division of labor is essential to avoid metabolic contradiction.

We here discuss "time" again. In our minds, we are governed by "absolute time" as suggested by Newton. Time appears to progress linearly. However, modern physics recognizes that time is more complicated.

Aristotle thought that movement existed, and time was derived from movement. This concept seems to approximate the "time" of life shown in circadian rhythms. Considering that we feel time by periodic movement, rhythmic biochemical reactions are important for time of life. Although pacemakers are required for life phenomena, human beings do not have precise clock-like watches. Naturally, it is thought that periodic biochemical reactions are used as a clock, which perceives the time.

During evolution, circadian clocks began with the rhythm of simple biochemical reactions [15]. Cyanobacteria possess a 24-hour clock that functions via enzymatic reactions [16]. However, these cases are exceptions. Circadian clocks mainly function through the negative regulatory feedback of transcription via the clock genes [17]. However, there are significant differences in clock genes between plants and animals. Although circadian clocks functioning with respect to the Earth's rotation have been conserved throughout evolution, the mechanisms and genes themselves have not been conserved. Considering these conditions, it is better to think that periodic biochemical reactions function as clocks rather than to consider biochemical reactions to be coordinated by clocks. The Earth's rotation is slowing due to the influence of the moon. Therefore, hundreds of millions of years later, days are thought to be 30 or more hours longer; thus, different internal clocks may be involved then.

Clock genes

As mentioned above, circadian rhythms involve a clock regulated by transcriptional negative feedback. CLOCK/BMAL1, transcription factors, binds to E-box hexanucleotides to activate the transcription of Per and Cry, which are the clock genes. The complex consisting of Per and Cry inhibits transcriptional activation by CLOCK/BMAL1. Subsequently, decreased Per and Cry activation in turn causes transcriptional activation. This cycle takes about 24 hours. Other clock genes adjust the clock by affecting the feedback system via E-box. Although small gaps among cells occur, it is surprising that this relatively precise clock is regulated by such a simple system.

Biological clock studies originally focused on the clock of the brain. They found that there is a master clock in the suprachiasmatic nucleus (SCN) of the brain, which exhibits strong autonomous oscillations over 24 hours. Consequently, it was understood that the brain clock, which is stimulated by light, controls the entire body. However, this understanding was revised because it was discovered that all peripheral cells have a clock. At present, it is understood that all cells have their own 24-hour clocks that function together as organ clocks, which collectively form an integrated clock through factors that synchronize organs. Therefore, synchronizers are important. The brain clock is generally synchronized by sunlight, which controls the peripheral tissues via the autonomic nervous system and endocrine system, which controls the clock of the entire body. In other words, light is the strongest synchronizer. This makes sense considering that circadian rhythms are based on the Earth's rotation.

However, the rhythm of the digestive system is reversed when the meal timing is reversed, indicating that the synchronizer of the digestive system clock is stronger than light. Meals have come to be understood as the strongest synchronizer of all organs. Therefore, meals synchronize the clocks of all organs present below the neck. This thought is rational considering that circadian clocks exist to help organisms obtain food in a timely manner. Thus, sunlight is used as a pacemaker to adjust the timing of meals. Furthermore, a study reports that meals synchronize the brain clock, too [18]. Meals may facilitate early recovery from jetlag in conjunction with light by adjusting the brain clock.

Examination at the gene level showed that approximately 10% of genes maintain a

rhythm in most organs including the liver, heart, and large intestine [19-21]. The peak time of gene expression varies by organ even for the same gene, which further indicates that each organ has its own clock. The clocks of the organs cooperate to control the functions of the entire body, which can be defined as good health. Although meals are a strong synchronizer of the organ clocks as mentioned above, the body clock is generally synchronized by sunlight as long as people keep eating at regular hours according to sunlight. However, it has become hard for many people to lead such lives in modern society. Many people have inverted meal timings similar to those in the animal experiment mentioned above. What should such people do? This is a problem of chrononutrition.

Clock gene abnormalities and lifestyle-related diseases

Experiments using clock gene knockout mice shows that the loss of clock gene causes not only behavioral disorders, but also metabolic disorders. A report which showed Clock gene knockout mice exhibited obesity and metabolic syndrome published in 2005 received much attention [22]. Furthermore, Bmal1 plays an important role in obesity [23, 24]

In addition to dysrhythmia, which was originally predicted, metabolic disorders in knockout mice revealed that the circadian clock is strongly linked to peripheral metabolism. Moreover, there is a report on familial advanced sleep phase syndrome due to mutations of Per2 in humans [25]. However, no associations between mutations of clock genes and metabolic disorders in human have been reported. Mutations in human clock genes are uncommon, indicating that clock genes may be essential for survival.

Irregular meal timing and lipid metabolism abnormalities

As mentioned above, lifestyle-related diseases are common in people who have irregular eating habits, such as shift workers. However, there is no evidence at the molecular level that meal timing itself causes metabolic disorders. Therefore, the influence of meal timing on lipid metabolism is not considered as significant, while the importance of well-regulated eating habits is recognized. Therefore, we examined the influence of meal timing using non-genetically modified animals. We developed a feeding protocol, in which the animals ate continually irrespective of time; although restricted feeding (e.g., feeding at noon only) causes day/night inversion in nocturnal rats, they get used to it. In 2009, we reported for the first time that irregular meals cause abnormalities in the circadian clock of the liver and increase blood cholesterol [26]. It was the first study demonstrating experimentally that irregular meal timing leads to metabolic disorders. It indicated that differences in meal timing cause cholesterol metabolism abnormalities even if the same quantity of food is provided. In the study, in order to make the rats lead irregular meal habits, a quarter of their daily feed was given 4 times a day, irrespective of night or day. We named this style “irregular eating.” Such timing can be used clinically for total parenteral nutrition. Although the weight of rats did not change as a result of irregular eating, levels of blood cholesterol, particularly that of VLDL (very low density lipoproteins)-cholesterol, increased significantly. Blood cholesterol levels increased to about 50 mg/dL due to the irregular meal timings. This was caused by the advanced shift of the circadian rhythm of the gene expression of CYP7A1, a rate-limiting enzyme involved in bile acid synthesis. Thus, orchestrated cholesterol metabolism did not occur and bile acid excreted in the feces decreased. Moreover, at that time, the rhythmicity of the clock gene DBP in the liver was advanced, which might be a major cause. In addition, clock genes, particularly Dec1 and Dec2, were susceptible to meal timings. These results indicate that well-regulated eating habits normalize the liver clock gene, normalize the rhythm of

CYP7A1 (which facilitates bile acid excretion), and that blood cholesterol levels are normalized due to normalized secretion of VLDL. In addition, apolipoprotein A-I, the main constituent protein of HDL (high density lipoprotein), is also under the control of DBP. This result indicates that irregular eating habits may reduce HDL. In other words, regular eating habits decrease "bad" cholesterol and increase "good" cholesterol.

The mice and rats with ad lib feeding eat 80% of the food during their active period in dark phase and the other 20% during their rest period. This raises the question of how mice and rats will react if they are forced to have tightly regulated eating habits, in which they eat no food during their rest period. A recent report indicates that diet-induced obesity is reduced only by tightly regulated eating habits [27]. This report importantly demonstrates that well-regulated eating habits actively contribute to good health rather than demonstrating that irregular eating habits are unhealthy.

Factors synchronizing peripheral clocks

The peripheral clocks, including the liver clock, maintain rhythmicity as a integrated clock of the whole body via the control of the SCN. As discussed earlier, the peripheral clocks are synchronized by meals independent of the brain clock, which is synchronized by light. In other words, there are presumably several factors that synchronize the peripheral clocks, including the nervous system, endocrine system (i.e., hormones), exercise (i.e., activity), body temperature, and eating behavior. To determine which factors synchronize the liver clock, rat primary cultured hepatocytes were treated with various hormonal factors.

Primary cultured hepatocytes obtained from rats kept on a 24-hour rhythm because the hepatocytes recognized the time even if the organism was dead. However, although spread monolayer cultures of hepatocytes, which were different from their original morphology, lost rhythmicity immediately, 3-dimensional cultures maintained circadian clock for long time [28]. These spherical hepatocytes were used for subsequent studies. Glucocorticoids [29], cAMP and cytokines which activate tyrosine phosphorylation changed gene expression of the clock genes in the liver and hepatocytes. Since it is already known that nutrients themselves, such as glucose, are synchronizers, we treated cells with single types of amino acids in high concentrations and found that many of them triggered various clock genes. We then focused on insulin, which is thought to be the most closely involved in the synchronization associated with meal timing. Insulin is a well-known hormone that fluctuates according to the meal timing [30-32]. However, experimental conditions and results vary widely among studies. Therefore, we aimed to resolve these discrepancies by performing a considerably large-scale experiment. The results show that insulin is a strong factor that synchronizes the liver clock [28]. The procedures of this experiment are described. First, insulin was added to hepatocytes with desynchronized clock gene expression, and synchronization by insulin was confirmed to ensure they had the same rhythm. In order to demonstrate that insulin is a synchronizer, we observed in real time hepatocytes obtained from transgenic rats that had a gene that linked luciferase to genes downstream of the clock gene promoter. The hepatocytes exhibited an obvious phase response curve to insulin. The phase response curve is a schematization to show that a given stimulus has a particular effect on clock resetting. If a phase response curve is found, it will demonstrate that the agent in question is a synchronizer. For example, in light therapy for treating sleep disturbances in "night people," although patients will become "morning people" by being exposed to strong light early in the morning, this therapy will be adverse if the patient is exposed to strong light at night. The effect of insulin in individual animals was examined using rats with streptozotocin-induced type I diabetes mellitus. The results revealed that the

liver clocks advanced in those diabetic rats due to insulin deficiency. Insulin administration exhibited a phase response curve, which presumably suggests that the abnormalities of the liver clocks in diabetic rats might be improved if treatment was performed during the active period when insulin was secreted. Although insulin administration during the active period (i.e., eating period) normalized the liver clock, insulin administration during the rest period hastened and worsened the liver clock. Considering these findings, we concluded that insulin is the synchronizer of the liver clock. Insulin does not synchronize the clocks of the cells or organs that do not generally respond to insulin, such as fibroblasts, the brain, and the lung, although it synchronizes the clocks of adipose tissue [28]. In other words, the clocks of the organs contributing to metabolic syndrome are entrained by insulin.

Eating itself as a synchronizer

The synchronization of the peripheral clocks, including those in the liver, are thought to be associated with the effects of meals when nutrients enter the body. However, synchronization also occurs via eating behaviors that stimulate the digestive system. Serum concentrations of glucocorticoid hormone (cortisol in humans and corticosterone in rats) secreted by the adrenal cortex shows circadian rhythm, and it is high just before the active period. Although the diurnal rhythm of insulin disappears when animals are starved, while glucocorticoid hormone continues to exhibit circadian oscillation., However, the circadian rhythm of glucocorticoid hormone is maintained when meals are administered orally, while the oscillations disappear when nutrients are administered parentally (i.e., not via the intestinal tract). On the other hand, the liver clock maintains its rhythmicity regardless of the administration route. In other words, the rhythmicity of glucocorticoid hormone from adrenal gland is entrained through nutrients entering the body orally [33]. Furthermore, resection of the jejunum abolishes the rhythmicity of the glucocorticoid, but not changes that of the liver clock [34]. These results show that food passing through the digestive organs or that getting absorbed per se synchronizes the clocks of some organs.

Food factors as synchronizers

As mentioned above, some nutrients act as synchronizers. Glucose is most important energy source that synchronizes circadian rhythms [35, 36]. It is reasonable that an energy source essential for survival has a rhythm-synchronizing function. Glucose also synchronizes the rhythm of cultured cells. These facts suggested that clocks of cultured cells could be synchronized by medium exchange alone. Actually, clocks can only be reset by medium exchange. These results unexpectedly gave us a problem. Although most researchers do not usually consider when culture media are exchanged, the timing of medium exchange may affect the results when the study subject possesses a rhythm. However, if we change the viewpoint, this problem may suggest a new concept of culture method, because metabolic contradiction is inefficient even in cultured cells. Cells cultured in media with fluctuated nutrient concentrations (“Rhythm culture”) must be effective when cultured cells are used for industrial purpose.

Amino acids alone can also exert synchronizing functions. It is reported that the rhythms of not only the liver, but also the SCN are synchronized when glucose and amino acids are administered to rats [18]. Meals may be a strong synchronizer for the brain. Another study also demonstrates that carbohydrate and protein act as strong synchronizers of the liver [37].

On the other hand, lipids have not been thought to be synchronizers, although high-fat diets seem to change the length (i.e., frequency) of one period (i.e., day) [38]. In mice, one period is generally approximately

23.5 hours; this increases to approximately 24 hours when they are fed a high-fat diet. On the contrary, a drug called clofibrate, which promotes lipid catabolism, counteracts this effect [39]. A pathway through PPARα seems to control the period of the rhythm.

Salt [40] and vitamin A [41] also synchronize the clocks. Resveratrol, a non-nutrient, affects the clocks [42]. Thus, there are many ingredients in food that control circadian clocks. Our everyday meals include many synchronizers. Consequently, daily meals provide synchronization stimuli.

Smart meal styles from an aspect of chrononutrition

Considering the findings mentioned above, we must determine what kinds of meal styles are good for our health, e.g., well-regulated eating habits that differ between daytime and night time (i.e., only eating during the active period but not during the rest period). Insulin will be secreted 3 times if 3 meals are eaten; the first insulin secretion is the most important (i.e., after long fasting). Eating breakfast after insufficient fasting may provide a weak reset effect. Midnight snacks alter the liver clock, making metabolism to function suboptimally.

Considering our biological clock, what should we eat? As mentioned above, meals act as bundles of synchronizers. Therefore, it may be sufficient if daily meals include carbohydrate and protein. Thus, it can be thought that we may take meals we usually eat without any specific limitation. However, it is important is to eat breakfast.

Is it acceptable to eat only at night due to daytime and night time inversion if eating habits are well-regulated in a sense? In humans, night eating syndrome, which causes lipid metabolism abnormality, has already become a problem. We performed restricted feeding such that rats were fed only at noon during the rest period and found that blood cholesterol levels increased remarkably. This experiment is still under investigation. Even if the timing of meals includes the accents (i.e., meal time and non-meal time) for 24 hours, metabolic disorders start occurring when the brain and peripheral clocks such as the liver clock are not orchestrated. A lack of coordination among the organ clocks also seems to lead to unhealthy condition, independent of the effects due to disturbances in the liver clock.

So therefore, should we strictly have 3 meals a day? The number of meals is thought to have increased from 2 to 3 after modernization. Eating more meals is reported to be better for health [43]. Although overeating is not good, having 4 or 5 meals a day is better than 3 or less because the index of obesity and blood lipid levels remain within normal range. Additionally, midnight snacking spoils the beneficial effect of the increased meal number.

We next think about “who” eats. Inactive lifestyles are now becoming a concern. Disuse syndrome and inactivity syndrome exist. Inactivity itself seems to cause poor health. Although inactivity syndrome is again attracting attention due to the Great East Japan Earthquake, the mechanisms of metabolic disorders derived from inactivity are not well understood. In Japan, bedridden elderly people so-called “Netakiri” have been a social and medical problem. We created bedridden “Netakiri” animal models for molecular biological study in order let bedridden people recover and to examine the biological reactions within their bodies. We found that this “Netakiri” rats showed various metabolic disorders, gene expression changes, and changes in the liver clock. It is possible that inactivity contributes to poor health by causing abnormalities in circadian rhythms including that in the liver. Furthermore, this result shows that physical activity itself may synchronize the organ clocks, e.g., that of the liver. “Netakiri” bedridden or inactive people may have disturbances in their body clocks. Therefore, if these disturbances are corrected, those people may be able to regain their health.

Because the timing of meals is usually linked to the sleep cycle, in order to correct

disorderly eating habits, it is necessary to improve the basic life rhythm. In any case, people should refrain from midnight snacking and have regular scheduled mealtimes; breakfast is especially important even if it is light. However, shift workers are forced to have irregular mealtimes. Hopefully, foods and drugs that exploit the known molecular mechanisms of biological clocks will be developed; such products would help shift workers. Considering that sleep disturbance is already treated with drugs, the prescription of drugs may be a practical method for treating metabolic disorders caused by disturbances in body clocks. It was recently found that some ligands of nuclear receptors (NR) can regulate the clocks [43], since some clock gene are NR such as Rev-erb and ROR.

A major problem faced when discussing eating habits from the perspective of chrononutrition is that details of one's own body clock cannot be understood. As long as we have no way to measure the biological clock, we cannot evaluate it, making it difficult to use in clinical settings. It is possible to collect blood every few hours, but this is impractical. The body clock was recently measured using hair follicle cells [44]; however, this is also impractical for use with the public and for use in clinical practice. Therefore, it is necessary to develop a method for measuring body clocks in a non-invasive and practical way. The oral mucosa is thought to be a substitute for hair follicle cells, but it is still impractical because mRNA of clock genes has to be extracted and measured. A simpler and easier method is required to utilize existing knowledge of chrononutrition completely. We are currently developing a smartphone application that estimates a person's body clock called "chrononutrition clock."

Conclusion and perspective

The first issue to be addressed in the future focuses on dysrhythmia underlying various diseases. Unless we clarify the usefulness and quantify the extent of how rhythm normalization prevents diseases, rhythm normalization cannot be used for prevention or treatment. Reduction of serum albumin secreted from the liver in elderly people is the second issue that must be overcome. Both albumin protein and mRNA have long half-lives but albumin gene exhibit circadian rhythms at the transcriptional level. The reasons for this apparently needless and hectic work remain unknown. Meal timing should help maintain normal albumin level because abnormalities occur in its transcriptional rhythm in the case of irregular eating. The third issue to be addressed is that the liver is a central organ of drug metabolism; disturbances to the liver clocks induced by irregular eating habits may cause abnormalities in drug metabolism, inhibiting expected efficacy and increasing unexpected side effects. Although it is known that drugs are affected by the timing of administration in chronopharmacology, drugs may not be effective unless the patient lives a regular life. It may be possible to improve the efficacy of drugs by having patients have regular meal timings, which would normalize the liver clock. We expect that the importance of chrononutrition as the basis for treatment timing, including chronotherapy and chronopharmacology, will become more important.

Nutritional sciences have traditionally focused on what a person eats. However, our future goal is to establish a well-regulated smart meal style called the "Smart Nutri Style" (SNS), which considers the way people eat or the meal style used (i.e., "5W1H of meals"). The French painter Delacroix stated that, "we work not only to produce but to give value to time." "To give value to time" means to give a meaning to life and to enrich human life. This wise remark should be revised to state that eating and metabolism not only to produce or maintain the body, but also to give value to time via circadian rhythms. Regular meal timings can help maintain our health and enrich our lives. By comprehensively considering molecular biological analyses, we would like to

advance nutritional sciences by increasing the understanding of living entities as integrated systems.

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Chapter 3.

Food Culture and the Structure of Disease

Harmonisation of Micronutrient Requirements in Europe

Nico van Belzen[a], Laura Contora[a], Fanny Rollina[a] and Christophe Matthysa[a,b],

Dietary Reference Values - DRVs (including average requirements) are quantitative reference values for nutrient intakes required to avoid deficiency and maintain function for healthy people. DRVs may be used for the assessment and planning of diets. The approaches used to derive DRVs, and their resulting values, vary considerably across countries and so far no evidence-based reason has been identified for this variation. Harmonization of the process of deriving DRVs is needed to align nutrition policy and public health strategies. In the context of this harmonization exercise, the EC Network of Excellence on EURopean micronutrient RECommendations Aligned (EURRECA) has been established to identify and develop methodologies to standardize the process of setting micronutrient reference values. EURRECA has derived a transparent approach for the quantitative integration of evidence on Intake-Status-Health associations and/or Factorial approach (including bioavailability) estimates. To facilitate the derivation of dietary reference values EURRECA is developing a process flow chart to guide nutrient requirement setting bodies through the process of setting dietary reference values.

Introduction

In Europe, micronutrient reference values have been established by (inter)national committees of experts and are used by public health policy decision-makers to monitor and assess the adequacy of diets within population groups. Nutrient requirements are traditionally based on the minimum amount of a nutrient needed by an individual to avoid deficiency, and is defined by the body's physiological needs. Alternatively the requirement can be defined as the intake at which health is optimal, including the prevention of chronic diet-related diseases. Both approaches are confronted with many challenges (e.g. bioavailability, inter- and intra-individual variability). DRVs are the complete set of nutrient reference values such as the adequate intake level, the lower threshold and upper intake levels. DRVs serve as a basis for nutritional educational programs, national and/or regional nutrition policies, and food regulations such as nutrition labelling [1, 2, 3, 4]. Dietary reference values based on scientific evidence are essential for the development of public health nutrition policies [5] as they are used by public health policy decision-makers to monitor and assess the adequacy of diets within population groups.

[a] ILSI Europe aisbl, Av. E. Mounier 83, Brussels, Belgium, publications@ilsieurope.be
(Please note that publications@ilsieurope.be is the email address to be printed (if any) in the publication. Correspondence before printing should be with nvanbelzen@ilsieurope.be, however)
[b] Present affiliation: University of Leuven, Oude Markt 13, Leuven, Belgium

The variation in micronutrient DRVs across countries [2)] can cause confusion among consumers, food producers and nutrition policy makers. More aligned information may change attitudes, thus influencing dietary behaviours and in turn potentially leading to a healthier population. They are used by health professionals to inform the lay public. For consumers, food industry and other stakeholders, this discrepancy is confusing.

In Europe, differences exist in the way micronutrient reference values are established and in micronutrient reference values themselves, even between neighbouring countries. For instance, vitamin D intake recommendations for 9-month infants in the Netherlands, Belgium and France are 5, 10 and 20-25 µg/day, respectively.

The variations can be partly explained by the fact that in some countries, nutrition societies are responsible for establishing the recommendations; while in other countries, it is the ministry of health, food safety authority, or advisory committee. The heterogeneity in nutrient recommendations is also in part due to the use of different approaches, changes in the approach in time and/or different data underlying them. Moreover, the terminology related to essentially the same nutritional concept (such as DRV) used in setting recommendations is heterogeneous [6)] (Table 1). The UNU (United Nations University) uses Nutrient Intake Value (NIVs) to refer to the set of reference values [7)], France and UK prefer the terminology Dietary Reference Values (DRVs), the USA uses Dietary Reference Intakes and the DACH (Germany, Austria and Switzerland) Reference values for nutrient intake.

In 2007, both the European Commission (EC) Research Directorate (DG-Research) and the Health and Consumer Affairs Directorate (DG-SANCO) have taken initiatives to harmonize the methodology of the derivation of micronutrient reference values. DG Research granted the EC Network of Excellence on EURopean micronutrient RECommendations Aligned (EURRECA) (www.eurreca.org) 13.2 million Euros for 5 years (2007-2012) to identify and develop methodologies to standardize the process of setting micronutrient reference values. EURRECA aims to identify and develop methodologies to standardize the process of setting micronutrient reference values. The consortium encompasses 35 partners from 17 countries and is coordinated by ILSI Europe. The purpose of this paper is to describe EURRECA's strategy (methods and potential applications) towards a uniform, transparent and evidence-based process of derivation of micronutrient dietary reference values.

Table 1 Terminology used for micronutrient reference values.

Organisation / country	Terminology
UNU	NIV – Nutrient Intake Value
USA	DRI – Dietary Reference Intake
F & UK	DRV - Dietary Reference Value
DACH	RVNI – Reference value for Nutrient Intake

EURRECA's approach and results

EURRECA has developed a Micronutrient Requirement Process Flow chart for use in deriving micronutrient reference values and setting reference values. This draft flowchart comprises 8 steps and is an iterating circle whose last step can be linked to the first, as improvement of public health is an on-going process. The steps are summarized briefly hereunder and described in detail elsewhere [8)].

The first step defines the problem in terms of nutrition and health. This includes identification of the health aspect, population group(s) and micronutrient(s) to consider. To identify which micronutrients are most in need of alignment, EURRECA has prioritized micronutrients based on the

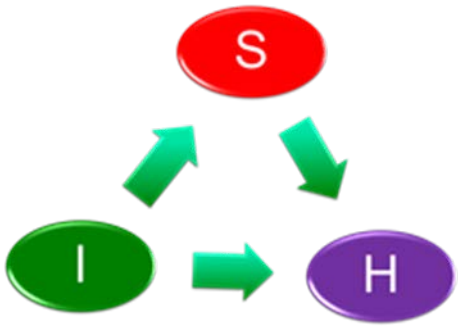
availability of new scientific evidence, public health relevance and heterogeneity of recommendations. Within the remit of EURRECA, 10 micronutrients have been included for further investigation: iodine, copper, calcium, folate, iron, selenium, zinc and the vitamins B12, C and D [9]. Population groups covered include infants (0-12 months), children and adolescents (1-18 years), adults (19-64 years), elderly (65+ years), pregnant and lactating women.

The second step serves to establish the infrastructure to address the question, including identifying the specific characteristics of the process to be followed, purpose, choice of committee members, and criteria on which to base the requirements and/or recommendations.

Steps 3 to 6 cover the physiologic basis for establishing DRVs. Step 3 establishes best practice methods; step 4 collects available data from systematic reviews; step 5 integrates the evidence into requirements; and step 6 derives DRVs from the requirements. Essentially there are two different approaches to these steps, as recently described [8]:

1) the 'classical' or 'factorial' approach (predictions, rather than measurements, of the requirements of groups or individuals, taking into account a number of measured variables (hence factors) and making assumptions where measurements cannot be made) focuses on physiological parameters such as body stores, data on micronutrient losses and maintenance and absorption/bioavailability measures. These parameters can be affected by homeostatic mechanisms, according to the level of intake, body status and also demographic factors.
2) In contrast, the 'association' approach (Figure 1) comprises health-related outcome measures, addressing the dose-response relationships between at least two of the following components: (i) health outcome (H), (ii) dietary micronutrient intake (I) and (iii) micronutrient status (S). EURRECA mainly focussed on the association (ISH) approach (Figure 1) and uses the factorial approach if necessary. For DRV estimation, the Intake-Health relation is of primary interest. However, as data may be scarce and heterogeneous, data on Intake-Status and Status-Health are also used.

Figure 1 Intake-Status-Health relationships



Legend: I= Intake, S= status, H= Health

(i) When assessing the Health parameter, a key decision is what functions / endpoints to take into account when setting DRVs. For example, vitamin D recommendations have traditionally investigated Intake and Status in relation to bone health [10]. However, recent data indicate that vitamin D may also be important in antimicrobial defence [11], cell proliferation, differentiation and apoptosis [12], cancer [13, 14] and cardiovascular disease [15]. Optimal health may require taking into account not only the classical functions but also the latest scientific evidence. As ultimate health outcomes may not always be easily accessible, e.g. in the case of cancer, biomarkers may have to be used as surrogate endpoints. The fast progress being made in areas like metabolomics is expected to provide increasing insight into optimal health that could be incorporated in micronutrient reference values.

(ii) On the Intake side, the methods used for nutritional adequacy assessment in Europe were reviewed and best practice guidelines related to the measurement of

dietary nutrient intakes were developed [16, 17]. Based on this methodology, EURRECA has systematically reviewed the literature on observational and intervention studies to collate the available data for the prioritised micronutrient for all age/life stage population groups in European countries. EURRECA has collated the data from these systematic reviews into a standardized database, including bibliographic details, methodological details, population characteristics, study groups details and outcome data. Several papers summarizing the extracted data will soon be available.

(iii) The Status parameter involves the use of biomarkers for micronutrient status. Biomarkers can be used to validate intake or identify deficiency. EURRECA published systematic reviews [18], eminence-based reviews [19] as well as best practice guidelines on biomarkers of micronutrient status. Although thousands of biomarkers have been identified in research, the large majority currently has limited value for the large population studies that would be desirable for micronutrient reference values. Common problems include uncertainty regarding what a normal range is and the invasiveness of the sampling procedure. Some biomarkers have particular issues; for instance, as subclinical inflammation increases ferritin, which in turn could mask iron deficiency [20]. Many biomarker activities have been or are being undertaken, e.g. Institute of Medicine (IOM), Biomarkers of Nutrition for Development (BOND), PASSCLAIM [21] and current projects within ILSI Europe. EURRECA is in contact will these projects to ensure a synergistic approach and avoid duplication of work.

Step 7 identifies the most appropriate policy responses, taking into account feasibility, (cost) effectiveness, and equality. Integrating the science of setting micronutrient reference values with other evidence bases and considerations may be beneficial during the decision making process to identify the most appropriate policy options. In particular, the policy options relevant to micronutrient recommendations can be mapped and the models of nutrient-related consumer behaviour change identified.

Step 8 implements the policy instruments. Policy makers are tasked with choosing policies that will maximize the likelihood of achieving a desired health outcome for the relevant population. Once a policy is implemented, impact assessment enables to assess changes in the nutrition situation that can be attributed in part or wholly to a nutrition policy. These steps may lead to the identification of a new problem, in case the cycle would be re-initiated. This step 8 is outside of the remit of EURRECA.

Tools developed by EURRECA

EURRECA developed several tools to assist in setting micronutrient reference values and deriving micronutrient recommendations [22].

The first set of tools mainly consists in interactive digital learning materials (see www.eurreca.org). The interactive digital learning material contains a sequence of interactive exercises, relevant information and associated theory modules, which guide the student through the design and analysis of evaluation studies. Most information in this module is presented in the form of animations, schemes or short texts to obtain an optimal balance between theoretical information and practical application. A large variety of exercises are used within the module. Potential audiences for these e-modules include university students, scientists, policy makers and members of industry.

The second set of EURRECA instruments comprises the development of a system for collecting different information resources. These information resources are collections of existing data and/or

knowledge; no new data are generated, although new analyses are undertaken on the collated data. This information includes best practice guidelines on intake assessment or biomarkers, interlinked webpages, online databases from systematic reviews. Among this set, the major tool to date is Nutri-RecQuest (http://www.serbianfood.info/eurreca/), a web-based search engine that allows comparison of existing micronutrient recommendations from 37 European countries /organisations and 8 non-European countries/regions. Nutri-RecQuest contains information on 29 micronutrients for infants, children & adolescent, adults, elderly, pregnant and lactating women; it lists over 20,000 recommendations in total [23].

Another major tool is the Best Practice Guidelines describing biomarkers for micronutrient status that have been developed on the basis of (i) expert-based assessment of the usefulness and application of key biomarkers [24] and (ii) evidence-based systematic reviews of the responsiveness of biomarkers to changes in exposure [25]. These guidelines contain measures of status and exposure for 20 key micronutrients, including cut-off values for key biomarkers of EURRECA priority micronutrients. The guidelines also describe the advantages and limitations of each measure.

The third set of instruments includes decision trees and frameworks. The Micronutrient Requirement Process Flow chart as described above is the best example. Furthermore, as micronutrient reference values should be regularly updated to reflect new scientific evidence, a decision tree on how to prioritise micronutrients for the purpose of reviewing their requirements has been developed by EURRECA9 (see step 1).

Key EURRECA messages

- Setting recommendations in a transparent, systematic way is **still difficult** - in most cases, there is a distinct lack of high quality studies
- The EURRECA **Micronutrient Requirement Process Flow Chart** provides a framework for deriving reference values
- EURRECA comprises:
  1. Integration of the association (I-S-H) and factorial approach (including bioavailability)
  2. Consideration of the policy aspects
  3. Development of a multiple micronutrients/health space
- EURRECA generated several tools, such as Nutri-RecQuest
- EURRECA established key collaborations to ensure its legacy

The future after EURRECA

To promote the sustainability of its outcomes, EURRECA widely disseminated the outcome of its work, including to the European Food Safety Authority (EFSA), national organisations that address micronutrient requirements, the World Health Organization (WHO), the Food and Agricultural Organization (FAO) and other stakeholders.

Moreover, as EURRECA funding will end in mid-2012, the EURRECA partners are planning to ensure the legacy of the methods, tools and information developed in the project, in collaboration with key stakeholders. After the funding period, the Network will continue working through 4 components:

- Early Nutrition Academy (ENA): ENA is developing a distance learning platform for the online Master course in Early Nutrition development of new e-modules related to this particular population group (e.g. iron in pregnancy)
- Centre for Evidence-Based Public Health Nutrition: to combine research and training (PhD). The first step will be the establishment of a consortium around public health nutrition.
- A close collaboration with Micronutrient Genome Project (MGP) / Biomarkers of Nutrition for Development (BOND): The Micronutrient Genome Project is a com-

munity driven project to facilitate the development of systematic capture, storage, management, analyses, and dissemination of data and knowledge generated by biological studies focused on micronutrient – genome interactions (eg databases). BOND is an initiative that aims at generating a renewable resource/tool that will be available to the full range of users (e.g. researchers, clinicians, programme and policy makers) involved in the global food and nutrition enterprise: the Query-Based System.

- EU Master of Advances in Nutrition: to create a proposal & test the realisation of a common Information Communication and Technology (ICT) platform for sharing of e-modules in Europe.

Acknowledgements

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A survey of dietary factors influencing a small proportion of obese people in Japan

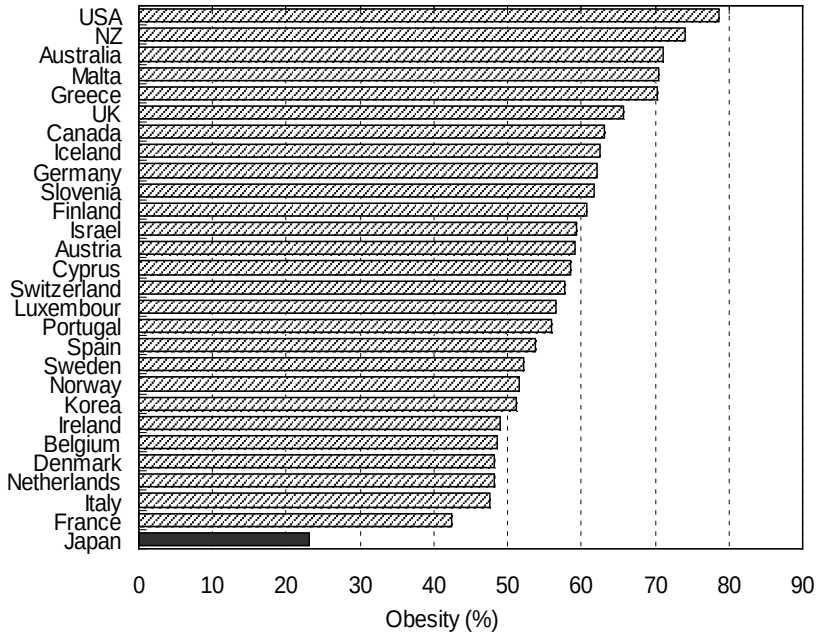
Naoki Midoh*

Introduction

The Westernization of food patterns is thought to have been associated with the recent increase in the prevalence of obesity in Japan; however, this prevalence has increased only in men, and now has begun to reach a plateau [1)]. Additionally, despite the increasing obesity prevalence, Japan has the lowest rate (obesity was defined in this study as a body mass index (BMI) of≥25, according to the Japanese criteria for obesity) among developed countries with comparable socio-economic profiles (Figure 1) [2)], along with the longest life expectancy as an average value of both sexes [3)]. Therefore, obesity seems to be a less significant health concern in Japan as compared to internationally; rather, the analysis of factors responsible for Japan's low obesity prevalence may help establish potential strategies to prevent and treat obesity.

Although a broad range of variables, including dietary habits, physical activity, and genetic background, should be taken into consideration when analyzing factors associated with the lower prevalence of obesity in Japan than in Western countries, the present study focused on Japanese dietary habits, which still retain unique characteristics in the face of recent westernization.

Figure 1 Prevalence of BMI≥25 in developed countries
Based on data from WHO Global InfoBase (2010) (Ref. 2)



* Processed Food Development & Technology Division, Knorr Foods Co., Ltd.

The Japanese diet has been linked to the low obesity prevalence in Japan, as demonstrated in numerous reports, but critical factors responsible for the health benefit remain to be clarified. The present study investigated available statistical data to explore dietary factors closely associated with obesity prevalence and discussed the results from a cultural perspective.

This study was carried out as part of the research theme designed by the "Japanese Dietary Habit and Obesity" Task Force (since 2006) of the International Life Sciences Institute (ILSI) Japan. This article is a revised version of a paper published in the Journal of Cookery Science of Japan [4].

Food consumption (patterns and levels) and obesity

In terms of the patterns and levels of food consumption, factors associated with obesity may include the overall meal size (weight), total energy intake, PFC (protein/fat/carbohydrate) balance, fat intake level, energy density, consumption levels of particular foods (e.g., meat, fish, vegetables, and added sugars), soft drink consumption, and portion size. Among these parameters, energy density and portion size vary across countries due to differences in the types of food consumed in each country, and it is thus extremely difficult to make an international comparison of these parameters. Additionally, there were no data available in the literature (except for the INTERMAP study [5] comparing four countries) that allowed international comparison of nutrient intake on a uniform basis. In this study, an international comparison of dietary intake was thus made with only two sets of data: the food supply data of the Food and Agriculture Organization of the United Nations (FAO) [6] and the soft drink production data of the Japan Soft Drink Association [7]. These data were adjusted for energy supply (a parameter linked to obesity) and car ownership [8] (a parameter showing an inverse correlation with physical activity) and analyzed for a possible correlation with obesity prevalence data provided by the WHO2). The causes of obesity are likely to differ between developing and developed countries [9], and, therefore, the present analyses targeted developed countries as defined by the International Monetary Fund (IMF) [10].

Table 1 Correlation between the prevalence of BMI≥25 and macronutrient supplies in developed countries
Analysis was based on data for the years in parentheses from WHO Global InfoBase (2002) [2], FAO STAT (2002) [6], and statistics on number of motor vehicles owned (1999-2004) [8]

Item	Correlation coefficient	Partial correlation coefficient			Japan's ranking (among 28 countries)
		Adjusted for energy	Adjusted for car ownership ratio	Adjusted for energy and car ownership ratio	
Energy (kcal/capita/day)	0.278	–	0.254	–	28
Amount (g/capita/day)					
Protein	0.295	−0.148	0.247	−0.058	25
Fat	0.224	0.183	0.227	0.031	27
Carbohydrate	0.123	−0.143	0.122	−0.017	22
Proportion (%)					
Protein	0.063	–	0.021	–	7
Fat	0.191	–	0.216	–	27
Carbohydrate	−0.206	–	−0.219	–	2

All p-values exceed 0.05.

Table 2 Correlation between the prevalence of BMI≥25 and food and drink supplies in developed countries Analysis was based on data for the years in parentheses from WHO Global InfoBase (2002)[2], FAO STAT (2002)[6], Annual Statistics Report on Soft Drinks (2002)[7], and statistics on number of motor vehicles owned (1999-2004)[8].

Item	Correlation coefficient	Partial correlation coefficient		Japan's ranking
		Adjusted for energy	Adjusted for energy and car ownership ratio	
Supply (g/capita/day)				(among 26-27 countries)
Total food amount	0.244	-0.018	0.146	26
Cereals	-0.027	-0.385	-0.238	14
Starchy roots	0.210	0.124	0.142	26
Meat	0.461 *	0.394	0.273	27
Fish, Seafoods	-0.325	-0.507 *	-0.484 *	2
Eggs	-0.481 *	-0.477 *	-0.574 **	1
Pulses	0.294	0.249	0.264	12
Vegetables	0.021	-0.027	0.132	14
Fruits	0.345	0.141	0.231	27
Sugar	0.297	0.363	0.345	25
Milk	0.282	0.186	0.180	25
Production (L/capita/day)				(among 23 countries)
Soft drink	0.274	0.126	-0.263	18
Carbonated drink	0.625 **	0.590 **	0.492 *	23

*: p<0.05, **: p<0.01

As the results, with regard to macronutrients, no significant correlation with obesity prevalence was observed for the consumption of energy as well as that of protein, fat, and carbohydrate in terms of the amount and proportion (Table 1). The balance between energy intake and energy expenditure affects body fat accumulation. The level of energy supply, which is substitute for energy intake, showed no significant correlation with obesity in the present analyses, indicating that, in developed countries, energy expenditure may have more of an impact on obesity than energy intake.

Regarding food supply, however, there was a significant correlation between the supplies of particular types of food and obesity prevalence. The supplies of fish/seafood and eggs showed a negative correlation with obesity prevalence, while the supply of carbonated drinks exhibited a positive correlation (Table 2). The results suggested that the consumption of these types of food might be associated with the low prevalence of obesity in Japan.

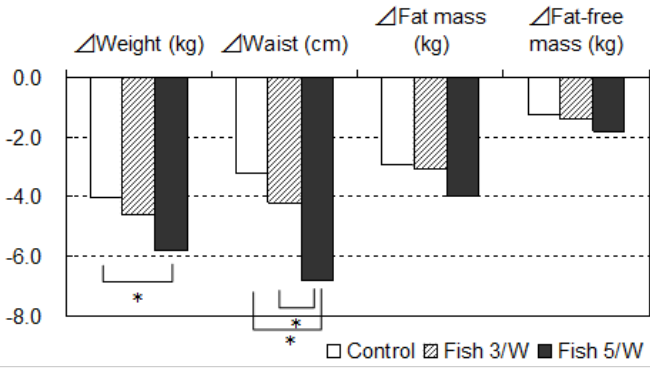
The following sections describe published research regarding the association between these food items and obesity, and discuss whether a dietary pattern consisting of such foods is contributory to Japan's low prevalence of obesity.

Fish/seafood consumption and obesity

Several studies have demonstrated that subjects on an energy-restricted diet achieved greater weight loss when they consumed lean fish compared with those without fish consumption. Ramel et al.[11], for example, carried out an eight-week parallel-group study, in which overweight or obese men and women on a 30% energy-restricted diet were allocated to one of three categories of fish consumption: no fish (control), 150 g of fish (cod) three times weekly (Fish 3/W), and 150 g of fish five times weekly (Fish 5/W) (Figure 2). The

Fish 5/W showed greater weight loss compared with the control group, along with a greater reduction in the waist circumference compared with the control and Fish 3/W groups. Despite being conducted under specific dietary conditions of energy restriction, the study suggested that a certain level of fish consumption could be effective in preventing obesity.

Figure 2 Effects of fish consumption on weight loss Based on Ramel *et al*.[11] (* P<0.05). Control: No fish consumption; Fish 3/W: Fish consumption three times weekly; Fish 5/W: Fish consumption five times weekly.



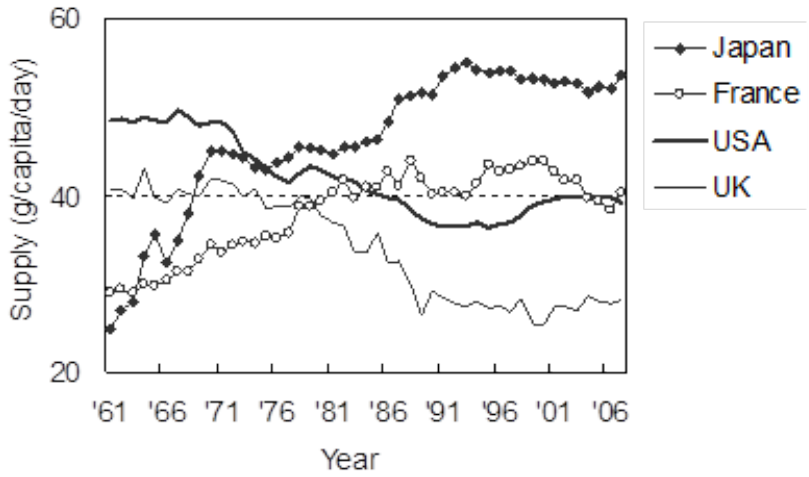
Previous reports have shown that histidine, an amino acid that is abundant in fish, can be converted to histamine to act to suppress appetite through the histaminergic nervous system[12]. Additionally, taurine and n-3 polyunsaturated fatty acids, which are also found richly in fish, have been found to promote weight loss, probably by facilitating lipid metabolism or inhibiting lipogenesis[13, 14]. These observations suggest that high-level consumption of fish is probably a factor responsible for Japan's low prevalence of obesity.

Egg consumption and obesity

Since egg consumption is thought to elevate blood cholesterol levels, there have been no reports with adequate experimental design showing whether egg consumption alone leads to weight loss or not. Therefore, it is unclear whether egg consumption is effective in preventing obesity.

An international comparison of egg supply trends [6] demonstrated that the supply decreased after 1970 in countries with higher obesity prevalence, including the United States and United Kingdom, and increased in countries with a relatively lower prevalence such as Japan and France (Figure 3). The decrease in egg supply in countries with a high obesity prevalence can be explained by the fact that egg consumption was once considered as a causal factor for elevated blood cholesterol levels, and, in countries with a high prevalence of obesity, i.e., a high prevalence of cholesterolemia, people tended to refrain from consuming eggs, resulting in a reversal of causality.

Figure 3 Trends in egg supply in Japan, France, the United Kingdom, and the United States. Based on data from FAO STAT[6].



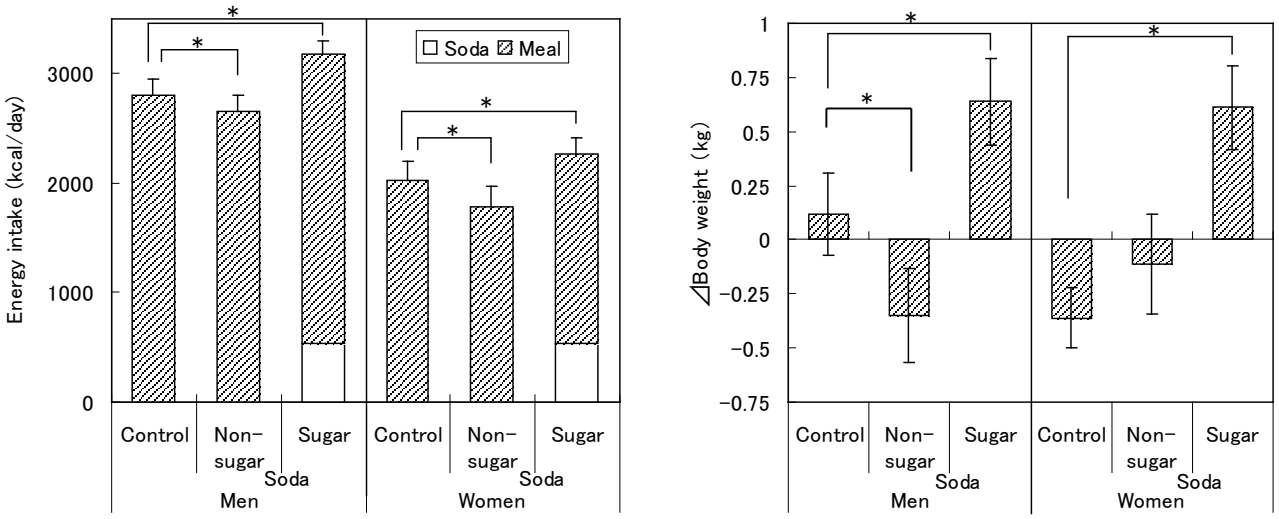
Therefore, the high-level consumption of eggs in Japan is less likely to be associated with the low prevalence of obesity. As for egg consumption, it has generally been accepted that a moderate consumption of eggs (1-2 eggs/day) has no impact on blood cholesterol levels, except for a specific population group with an increased susceptibility to dietary cholesterol-induced hypercholesterolemia[15].

Carbonated drink consumption and obesity

Regarding carbonated drinks, carbonation itself does not appear to bring about obesity. Rather, as we usually experience, carbonic acid in these drinks has been shown to enhance the sensation of satiety and thereby act to reduce energy intake from food ingested after carbonated drink consumption[16]. Considering also that carbonation is unlikely to promote body fat accumulation, the positive correlation observed between carbonated drink production and obesity prevalence may reflect an increased intake of sugars that provide energy. If there was classified data as sugar-sweetened drinks, the high positive correlation, as is the case with carbonated drinks, would be observed.

The consumption of sugar-sweetened drinks does not reduce energy intake from meals and, thereby, adds extra calories that contribute to weight gain, an observation which has been reported by numerous authors including Tordoff et al.[17], as described below. To clarify the link between sugar-sweetened drink consumption and obesity, they conducted a three-period cross-over study, in which participants (men and women) were assigned to 1,135 g/day of sugar-free soda sweetened with a low-caloric sweetener, sugar-sweetened soda, or no experimental drink (control) for three weeks each in a cross-over fashion (Figure 4). The participants showed an increase in total energy intake from food and drink sources as well as in body weight when they consumed the sugar-sweetened drink, whereas they showed a reduced total energy intake (in both genders) and weight loss (in men) during the sugar-free drink period. The results suggest that an increased energy intake due to sugars from caloically-sweetened drinks may lead to weight gain.

Figure 4 Effects of non-sugar vs. sugar-sweetened soda consumption on energy intake and body weight. Based on Tordoff et al.[17] (Mean ± SE, * P<0.05).

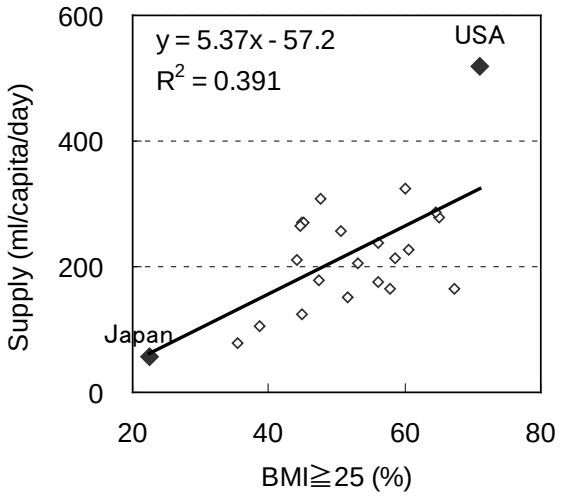


A possible reason for weight gain associated with sugar-sweetened drink consumption is that such drinks give rise to less of a feeling of satiety than other isocaloric foods, resulting in an increase in energy intake to a level higher than that required normally. A sense of satiety is caused by: (1) nutrients produced by the digestion of food, (2) food-induced gastric distension, (3) food-induced mechanical stimuli of gastric mucosa and the intestinal tract, and (4) masticatory movements[18, 19]. Being ingested in liquid form, sugar-sweetened drinks require no chewing and have a shorter gastrointestinal transit time, causing less gastric distension and weak mechanical stimulation of the gastric mucosa and intestinal tract, responses which are thought to be associated with lower satiating effect of these drinks relative to their energy contents, leading to an increase in total daily energy intake.

The positive correlation observed between carbonated drink consumption and obesity prevalence remained after adjusting for energy supply, suggesting that factors other than increased energy intake may exist concerning the drinks to affect the likelihood of developing obesity (Table 2). A candidate factor for this link is fructose. Unlike glucose, fructose does not increase blood glucose levels and, thereby, causes no rise in the blood levels of insulin or leptin. Moreover, fructose is rapidly metabolized to acetyl-CoA without being mediated by phosphofructokinase, a limiting enzyme that regulates glucose metabolism, and brings about an increase in circulating triglycerides[20] that are consequently incorporated into lipogenesis to accelerate body fat accumulation. An earlier report also demonstrated a reduction in intracerebral malonyl-CoA (a substance that functions as a signal to suppress food intake) after fructose administration[21].

Suppose that carbonated drinks have an energy content of 46 kcal/100 g[22] and non-caloric "diet" sodas account for ≈40% of these drinks[23], the difference in carbonated drink consumption between the United States, a country with a widespread obesity epidemic, and Japan (≈460 ml/day) represents an energy intake difference of ≈130 kcal/day (Figure 5), which, if converted entirely into fat in the body, is estimated to add ≈6.5 kg of body fat annually. Although sugar-derived energy is not fully converted into body fat, a proportion of the energy can be partitioned to body fat storage, a concept which may explain the increased prevalence of obesity in the United States. These observations strongly indicate that the low-level consumption of sugar-sweetened drinks is a contributory factor to Japan's low obesity prevalence.

Figure 5 Association between carbonated drink production and the prevalence of BMI≥25 in developed countries
Based on data from WHO Global InfoBase2) and Annual Statistics Report on Soft Drinks[7]



An excessive consumption of sugar-sweetened drinks is likely to promote obesity, yet, these drinks may help provide energy in cases where increased energy intake is desirable (e.g., exercise and fatigue), and can therefore be used effectively as a good source of energy under such conditions, without having any concerns about their impact on obesity. Drink manufacturers

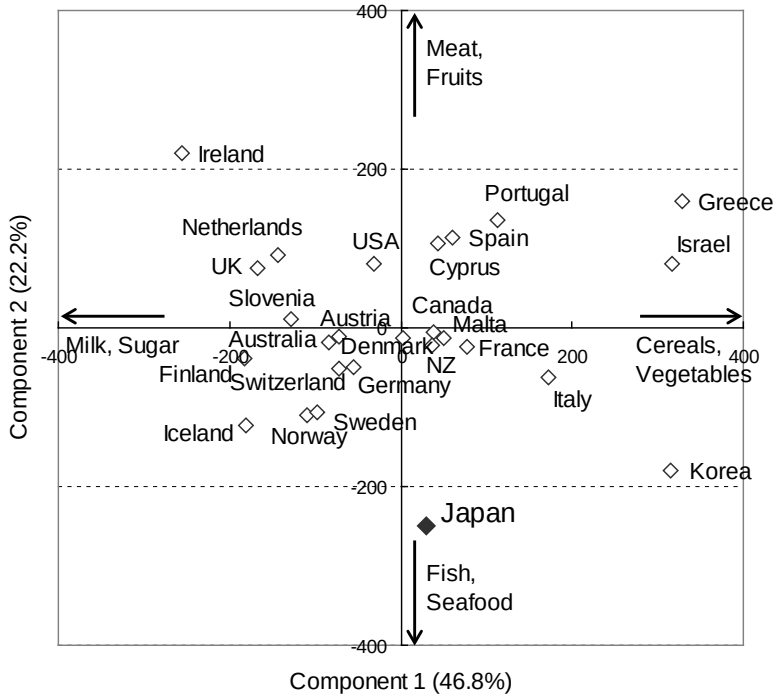
have shifted their distribution to non-caloric "diet" drinks, a possible approach by which the manufacturers can address the health issue of adulthood obesity.

Japanese diet: A discussion from a cultural viewpoint

An international comparison of food supply patterns (Figure 6) shows that the Japanese diet appears to occupy a distinctive position compared with that of other countries, with a characteristic dietary pattern of high fish and low sugar-sweetened drink consumption, two dietary factors that were found to be linked to obesity in this study. Then, why has Japan developed such a food culture?

Food culture is generally considered to be determined by the local climate, because native flora and fauna vary across regions depending on climatic variables. The southeast region of the Eurasia continent, including Japan, has warm, humid climatic conditions. Under such environmental conditions, as wet-paddy rice agriculture and fisheries evolved, rice and fish became major dietary components. These food items were also used as ingredients to produce seasonings such as soy sauce and fish sauce. Along with the cultivation of tea (a crop suitable for warm, humid climatic conditions) and access to water of good quality, green tea became a major drink consumed on a daily basis. Additionally, in Japan, owing to the geographic environment, in which mountainous areas account for 70% of the land that is surrounded by sea, and the effects of the law on meat taboo issued in AD 675, pig farming that usually coexists with rice cultivation did not pervade across the country, resulting in a dietary pattern with a higher proportion of fish compared with other countries. The traditional Japanese meal pattern (cooked rice, soup, and one or three kinds of main/side dish) led to the customary consumption of soup[24-26]. Another characteristic feature of the Japanese diet is increased soybean consumption. Soybeans became a common food item probably as a satiating plant food alternative to animal protein foods.

Figure 6 Principal component analysis of food supply in developed countries Analysis was based on data from FAO STAT (2002)[6] for cereals, starchy roots, meat, fish/seafood, eggs, pulses, vegetables, fruits, sugar, and milk.



On the other hand, under cold, dry climatic conditions, for which the cultivation of rice (food that requires only boiling to bring forth its full flavor) was not suited, wheat cropping and pastoral farming of grass-fed sheep and cattle evolved, and bread, meat, and dairy products thus became major dietary components. Dairy products, such as butter and cheese, were commonly used as a flavor enhancer, and wine and beer were consumed due to limited access to quality water (these drinks were produced through fermentation to add improved shelf life and purity for the purpose of securing a safe supply of fluid/water). These observations suggest a basis for the markedly diverse food cultures that developed in Japan and Western countries [24-27].

As mentioned above, the high levels of fish consumption among Japanese are largely due to an abundant supply of marine fishery resources, while contributory factors that curbed the consumption of sugar-sweetened drinks include rice, soup, and green tea consumption, characterized as follows: (1) a rice-based diet is generally low in salt and needs supplemental salt, and cooked rice tastes rather bland and complements foods with a salty taste; (2) consumption of foods with a high water content, such as cooked rice and soup, reduces fluid intake from drinks; and (3) plain green tea has traditionally been consumed in Japan.

Possible reasons for the high consumption of sugar-sweetened drinks in Western countries may include: (1) toasted bread has a smoky flavor and complements a sweet flavor; (2) bread has a limited water content and is thus served with drinks; (3) drinks with a sweet flavor, such as cow's milk, wine, and beer from ancient times, followed by coffee, and tea, have traditionally been consumed in Western countries, and these drinks are readily substituted with sugar-sweetened drinks including carbonated drinks[27].

Japanese food culture: Future view

As described above, dietary/cultural factors associated with Japan's low prevalence of obesity probably include high-level consumption of fish, rice, soup, and green tea. Among these food items, the high-level consumption of rice, soup, and green tea is considered to be associated with the low-level consumption of sugar-sweetened drinks. Then, the question arises as to whether this food culture will remain unchanged in the future.

Trends in the supplies of relevant food items[6, 28] show that the supply of tea (raw material) has tended to increase since 1980, whereas those of fish, rice, and miso paste (as an indicator of soup consumption) have consistently decreased (Figure 7). Among various types of soft drink, an increased supply was observed recently for sugar-free tea drinks and bottled water, a trend that reflects consumers' health concerns, but not for sugar-sweetened drinks[7, 29, 30] (Figure 8). The consumption of sugar-sweetened drinks is less likely to increase in the future considering the fact that non-caloric "diet" drinks already account for a large part of the sweetened soft drink market. That is to say, from the standpoint of obesity prevention, it may be said that both positive and negative changes are on-going. Appropriate strategies need to be undertaken to curb the negative change, especially decline of fish consumption. If fish consumption will be maintained, the consumption of rice and soup will also be maintained due to its suitability to the taste of fish. This would contribute to suppress further the consumption of sugar-sweetened drinks, though the increase of the consumption of sugar-sweetened drinks has not been found.

Conclusion

Japan has an extremely low prevalence of obesity among developed countries. The author speculated that the reason for the difference lies in the Japanese dietary pattern: high-level consumption of fish and low-level consumption of sugar-sweetened drinks. The results suggest that marked fish

consumption among Japanese is substantially attributed to an abundant supply of marine fishery resources, while a lower prevalence of the regular consumption of sugar-sweetened drinks compared with other developed countries is associated with the Japanese food culture characterized by the consumption of rice, soup, and green tea.

Although there has been a controversial suggestion that the Japanese diet is high in sodium and low in calcium[31], Japanese have an extremely low prevalence of obesity and high rates of life expectancy in the world, indicating relatively optimal dietary patterns. However, recent trends in food supply predict possible changes in Japanese dietary patterns in the future, suggesting the importance of maintaining the fish consumption especially.

Figure 7 Trends in food supply in Japan
Based on data from FAO STAT[6] and food balance sheet[28].

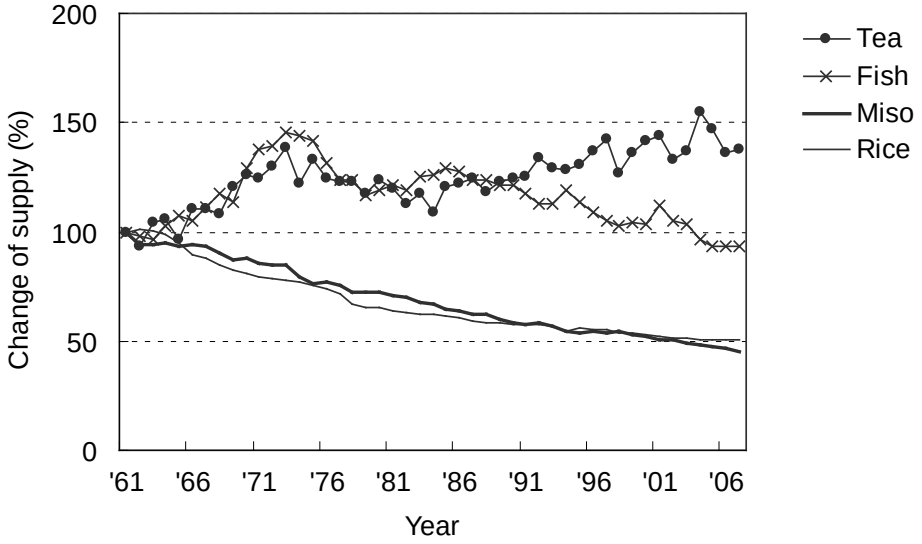
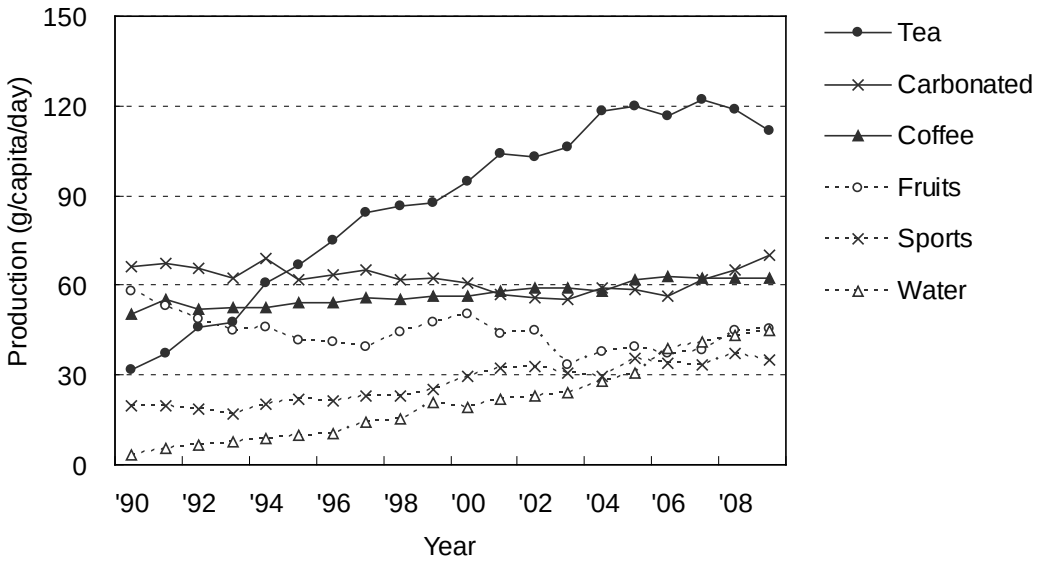


Figure 8 Trends in drink production in Japan
Based on data from Annual Statistics Report on Soft Drinks[7, 30] and Annual Statistical Data on Liquor & Food Industries[29].



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Nutrition for Middle aged and Elderly in China

Jian Zhang* and Junshi Chen*

Abstract

To describe the status and trends of foods, dietary nutrients intake and prevalence of under nutrition diseases among middle-aged and elderly population in China based on the data from 2002 China Nutrition and Health Survey and 1992 National Nutrition Survey. The average intake of cereals, vegetables, animal foods, fruits and legumes is 416.2g, 290.3g, 130.2g, 40.4g, 16.5g for middle-aged (45-59y) people, and 357.5g, 290.3g, 121.8g, 40.2g, 17.0g for elderly (aged over 60y). The average energy, protein, fat and carbohydrate intake for middle-aged is 2309 kcal, 67.9g, 78.3g, 326.2g, and for elderly is 2025.1 kcal, 61.4g, 71.0g, and 280.1g respectively. Middle-aged and elderly people living in urban area intake more animal foods, vegetable oil but less cereals, vegetables than those living in rural areas. The intake of dietary fat and retinol are higher while intakes of energy, carbohydrate are lower for urban middle-aged and elderly people. With the economic transition, changes happened in foods and nutrients intake. The obvious changes in foods consumption are reduction in cereals, dark & light vegetables intake while increase in edible oil, animal foods, fruits and milk intake and thus decrease in total energy intake per capita while the percentage of energy supplied by dietary fat, particularly animal fat, increased. Compared to that in 1992, the prevalence of under-nutrition has a reduction of approximately 30% in urban and 20% in rural area. However, although the prevalence of anemia has more than 30% reduction for the elderly in urban area, it doesn't achieve significant improvement in rural area. For rural elderly people, particularly elderly women, the reduction of anemia prevalence is less than 5% during these ten years. With the rapid economic development, it achieves a substantial improvement in the nutrition status for middle-aged and elderly in China. However, the nutrition related chronic diseases have also become more and more serious. The national prevalence of obesity, hypertension, diabetes, hypercholesterolemia, hypertriglyceridemia is 10.2%, 29.3%, 4.3%, 4.7%,15.7 for middle-aged people and 8.9%,49.1%,6.8%,6.1%,14.8% for the elderly. With the increasing percentage of energy supplied by dietary fat, odds ratio of obesity, diabetes, hypercholemia increases. Therefore, due to the unbalanced economic and social development in urban and rural area, China also faces double burden issue. We will fight against under-nutrition problems such as elderly anemia and at the same time put more efforts to prevent over-nutrition and its related chronic diseases such as obesity, diabetes, dyslipidemia, etc., particularly for those living urban area.

Introduction

The fastest growing population segment is the elderly in most industrialized countries and this trend has also appeared in China. Actually, over the past 20 years, the aging population increased by 3.02 million every year and in 2005, the ratio of aging population (over 60 y) has reached 11.0% -

* Institute of Nutrition and Food Safety, Chinese Center for Disease Control and Prevention

approximately 143 million and it is anticipated that the elderly population will reach 173 million and in 2050, three out of 10 Chinese will be the seniors. Thus although China achieves great success in controlling the total population by one-child policy, it now has to face lots of challenges caused by aging.

Good nutrition and health status not only forms the foundation for the social and economic development, but is also the goal of the country's social and economic development. The nutrition status of middle-aged and the elderly people is important to their productivity and health. Many countries in the world have been conducting regular surveys on the status of nutrition and health among the people and release the results in time. Based on the survey results, relevant social development policies have been formulated and evaluated accordingly in a view to improve the status of nutrition and health among the people and to promote the coordinated development of social economy.

In China, three national surveys on nutrition were conducted respectively in 1959, 1982 and 1992, and several epidemiological surveys on non-communicable diseases such as hypertension, diabetes had also been carried out. Although these surveys do not only concentrate on middle-aged and aging population, lots of valuable outcomes had been obtained for understanding of urban and rural middle-aged and elderly residents on dietary patterns, nutrition status, epidemiological characteristics and trends of the relevant chronic degenerative diseases. As Chinese economy is developing at a fast pace in recent years, the dietary pattern and lifestyle is also changing rapidly. In order to timely understand the recent dietary structure and the status of nutrition and health among middle-aged and elderly people, and provide the scientific basis for formulating relevant state policies to solve problems caused by aging, ensuring China's well-being, sustainable development, Chinese Nutrition and Health Survey was conducted under the joint leadership of the Ministry of Health, Ministry of Science and Technology and the National Bureau of Statistics from August to December, 2002.

This was China's first comprehensive survey ever in the field of nutrition and health. It has systematically integrated several previously separately organized surveys on nutrition, hypertension, diabetes, etc. into one survey, and it has increased some new and relevant indicators and contents taking into account of the status of social economic development. The following will review the major findings from this survey focus on middle-aged and elderly people.

Methodologies and contents

This survey covered China's 31 provinces, autonomous regions and the municipalities directly under the Central Government (excluding Hong Kong and Macao Special Administrative Regions and Taiwan), and it has exhibited good representatives of the nation, taking into account of the different regions in China. This survey was scientifically designed with abundant contents, sufficiently embodying the advantage of cooperation among the multi-sectors and the application of multi-disciplinary scientific knowledge and by so doing, it has not only saved a large number of manpower and the material resources, but has also avoided the overlap of survey contents and indicators, laying the foundation for further analysis on the inter-relationships among different factors.

The method of multi-steps cluster sampling was adopted, 71,971 households (24,034 urban households and 47,937 rural households) were chosen from 132 counties (districts, cities) of China's 31 provinces, autonomous regions and the municipalities directly under the Central Government, 243,479 persons (68,656 persons in the cities and 174,823 persons in the rural areas) were chosen as samples, among them, 16,903 middle-aged (45-59y) persons, 8054 males, 8854 females, and 9989 aging persons (60y-), 5025 males, 4964 females.

The survey composed of 4 parts: general health questionnaire, basic physical examinations, biochemical measurements and dietary survey.

1. Foods consumption and Nutrients Intake

(1) Average Food Intake

With great efforts on agriculture production and investment on food industry, foods supply has the varieties of foods including many imported foods are available on the market with sufficient quantity. This is particularly obvious in urban area where people's income increase significantly in recent years. The nationwide average intake of cereals, vegetables legume, fruits, animal foods, dairy products, edible oil and salt is 416.2g, 290.4g, 16.5g, 40.4g, 130.1g, 25.9g, 43.5g, 12.9g for middle-aged people and 357.5g, 253.3g, 17.0g, 40.2g, 121.8g, 44.5g, 38.7g, 10.9g for the elderly.

However, due to the unbalanced development, the food consumption pattern is quite different between people living in urban and rural area. The cereals, vegetables, salt intake of rural middle-aged people and the elderly is higher than that of urban while animal foods, dairy products and fruits intake is higher in urban (Table 1).The changes of foods intake is quite different in subgroups. Comparing with the data of 1992, the average milk consumption for urban middle-aged people increased from 30.3g to 59.5 g, while in rural, it just increase from 4.1 g to 8.9g

Table 1 Average Food Intake of Middle-aged and the elderly

	45-59 y			60y -		
	National	Urban	Rural	National	Urban	Rural
Cereals	416.2	337.4	456.3	357.5	303.8	397.9
Vegetables	290.4	256.8	307.5	253.3	236.3	266.0
Legume	16.5	15.6	17.0	17.0	16.1	17.7
Fruits	40.4	64.0	28.4	40.2	66.4	20.6
Animal foods	130.1	172.9	108.4	121.8	159.9	93.1
Dairy products	25.9	59.5	8.9	44.5	86.5	13.0
Edible oil	43.5	42.4	44.1	38.7	40.2	37.5
Salt	12.9	10.1	14.3	10.9	9.2	12.2

Table 2 Nutrients Intake of Middle-aged and the elderly

	45-59 y			60y -		
	National	Urban	Rural	National	Urban	Rural
Energy	2309.4	1988.1	2472.7	2025.2	1839.5	2164.6
Protein	68.0	65.2	69.4	61.4	61.3	61.5
Fat	78.3	81.2	76.8	71.0	77.0	66.4
Carbohydrate	326.2	243.4	368.3	280.1	222.0	323.8
Calcium	408.7	432.5	396.6	396.9	444.2	361.4
Iron	24.2	22.5	25.1	21.6	21.2	22.0
Zinc	11.6	10.8	12.0	10.3	10.0	10.5
Retinol Equivalent	489.8	527.9	470.5	460.3	530.3	407.9
Vit B1	1.1	0.9	1.1	0.9	0.8	1.0
Vit B2	0.8	0.8	0.8	0.7	0.8	0.7

(2) Nutrients intake status

Nutrients intake is closely linked to foods consumption and show the similar pattern. The nationwide average intake of energy, protein, fat, carbohydrate, calcium, iron, zinc, retinol equivalent is 2309 kJ, 68.0g, 78.3g, 326.2g, 408.7mg, 24.2mg, 11.6mg, 489.8ug for middle-aged people and 2025kJ, 61.4g,71.0g, 280.1g,396.9mg, 21.6mg, 10.3mg, 460.3ug for the elderly. The energy, carbohydrate, iron intake of rural middle-aged people and the elderly is higher than that of urban while dietary fat, calcium and retinol equivalent intake is higher in urban (Table 2)

The protein intake is lower than that recommend and the protein intake of urban people is lower than that of rural. This suggest that with the improvement of protein quality (increasing the percentage of animal source), the recommended dietary protein RNI could be reduced.

Dietary fat is important for providing energy and promotes the absorption of lipid vitamins. However, due to the increasing consumption of animal foods and usage of vegetable oil for its character of improving food taste, dietary fat intake is increasing significantly. Data from 1992 shows that the percentage of energy provided by dietary fat is 22%, while in 2002, this ratio reached 30%, for urban middle-aged and elderly people, it even reached 36.8% and 37.7% (Figure 1).

(3) Nutrients Supplement consumption

Physiological, psychological changes make elderly people most susceptible to many kinds of diseases and they pay more attention on their health. For older people, the function of digestion and absorption is declining and this has negative effects on their foods consumption and nutrients usage. At present nutrients supplement taking is more and more acceptable among elderly Chinese. It shows that nutrients supplements consumption is much higher among urban elderly people than that of rural people and the middle-aged people in urban because of higher living standard and the strong health awareness (Figure 2).

2. Anthropometric measurements

The nationwide average height is 165.0cm, 162.8 cm for male middle-aged and the elderly people and 154.0 cm, 150.8 cm for the females. The height of urban middle-aged and elderly people is higher than that of rural people both in males and females (Figure 3).

Figure 1 Ratio of Energy provided by protein, fat and carbohydrate

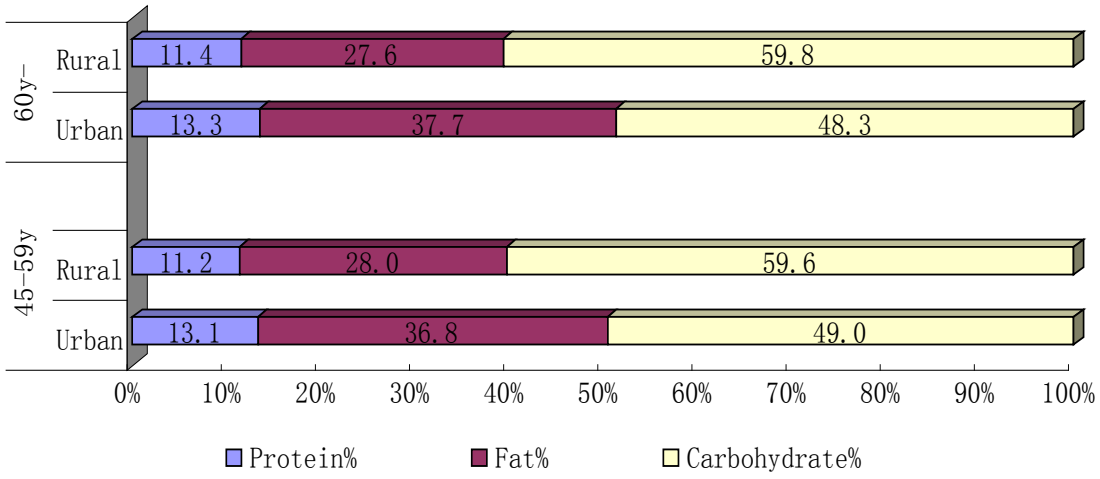


Figure 2 Ratio of nutrients supplements usage in middle-aged and elderly people.

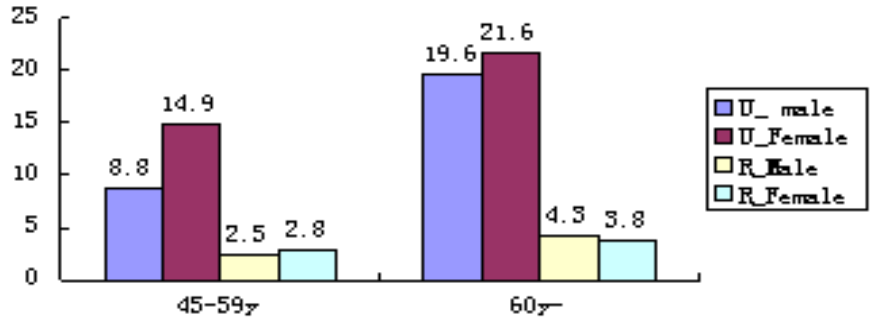


Figure 3 The height of middle-aged and elderly Chinese

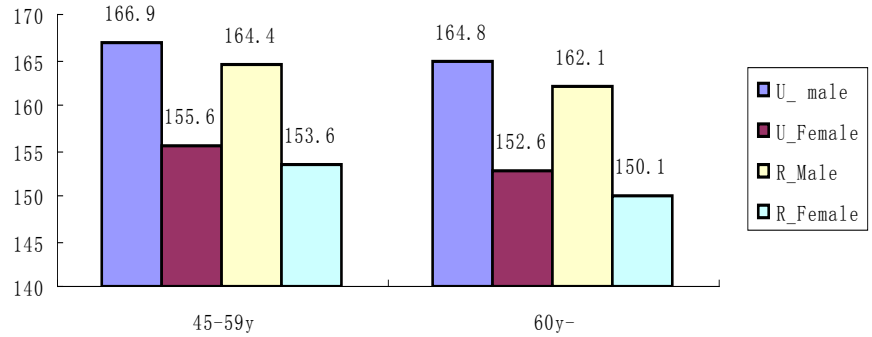
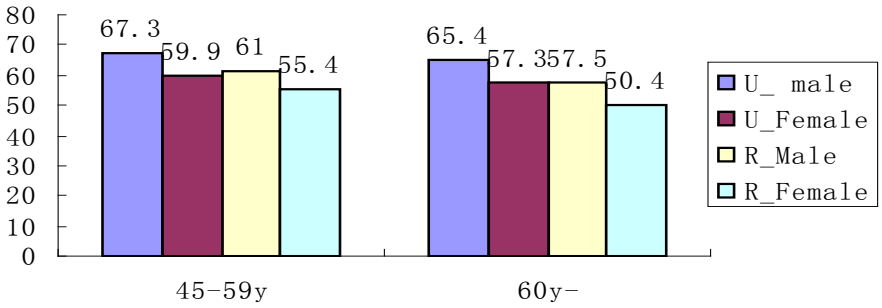


Figure 4 The weight of middle-aged and elderly Chinese



The nationwide average weight is 62.7 kg, 59.5 kg for male middle-aged and the elderly people and 56.6 kg, 52.4 kg for female. The weight of urban middle-aged and elderly people is higher than that of rural people both in males and females (Figure 4).

Body mass index (BMI) is a commonly used indicator to estimate the population's nutrition status. Overweight and obesity have been proved to have strong relationship with many chronic degenerative diseases such as hypertension, diabetes, dyslipidemia, etc. The nationwide average BMI is 22.9, 22.4 for male middle-aged and the elderly people and 23.7, 22.9 for the females. The BMI of middle-aged and the elderly in urban is higher than that of those in rural while the BMI of the females is higher than that of the males. For middle-aged and elderly people, BMI is closely linked to the variation of body weight. With to the increasing of body weight, the BMI of middle-aged and elderly population also increased when comparing to the data of 1992 both urban and rural people (Figure 5).

Figure 5 The BMI of middle-aged and elderly Chinese

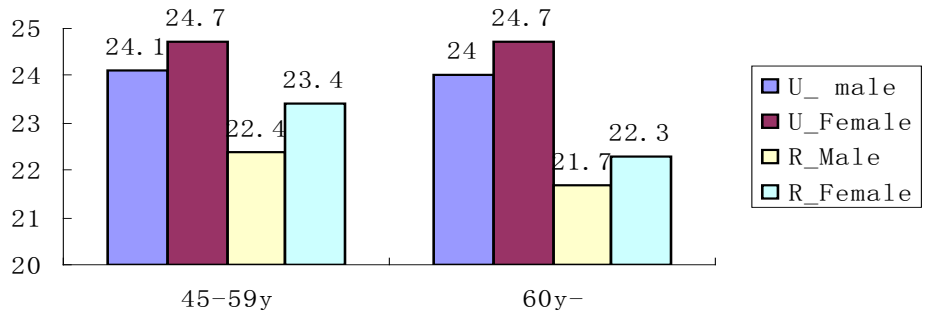


Table 3 Rate of under-nutrition for middle-aged and the elderly

		National			Urban			Rural		
		1992	2002	Change	1992	2002	Change	1992	2002	Change
45-59y										
	Subtotal	8.6	5.5	-36	4	2.8	-30	10.3	6.5	-36.9
	Male	7.5	5.3	-29.3	4.1	3.5	-14.6	8.8	6	-31.8
	Female	9.6	5.7	-40.6	3.8	2.4	-36.8	11.8	7	-40.7
60y-										
	Subtotal	17.4	12.4	-28.7	9.6	5.4	-43.8	20.3	14.9	-26.6
	Male	16.1	12.5	-22.4	9.1	6	-34.1	18.8	14.9	-20.7
	Female	18.7	12.2	-34.8	10.1	4.9	-51.5	21.9	14.9	-32

3. Under-nutrition related Diseases

Based on the BMI, of which the cut-off point is 18.5, the under-nutrition rate was estimated. The nationwide under-nutrition rate is 5.5% and 12.4% for middle-aged and the elderly people. The rate of rural middle-aged and elderly people is much higher than that of urban people. For elderly people, the under-nutrition rate in rural area is nearly three times of that in urban (Table 3).

As a developing country, China has suffered shortage of foods supply and thus the under nutrition problem for a long time. With rapid development of economic and great efforts made by government to eradicate poverty, the foods insufficiency problem has been largely solved and there achieve a great improvement in nutrition. Comparing with the data of 1992, the rate of under-nutrition has a significant reduction for both middle-aged and the elderly, in particular, for middle-aged rural and elderly urban females.

Anemia is also a public health problem in developing countries and lots of works have been done to reduce the prevalence of anemia among children and young women in China. However, anemia problem is also serious in middle-aged and elderly people which get less than attention.

The nationwide anemia rate is 23.1 %, 28.5 % for middle-aged and the elderly. The anemia rate in rural area is higher than that in urban area and the rate of female is higher than that of male. Comparing with the data of 1992, there has a significant reduction for urban middle-aged people, -19.6% for male, -27.5% for female, and for urban elderly people, -30.2% for male, -33.7% for female. Quite different is that there is little change for rural people. For middle-aged people, there even has a slight increase of anemia rate for male and female and the reduction for the rural elderly is

much lower when comparing to that of urban elderly people (Table 4).

Comparing to the data of under-nutrition rate based on BMI, the reduction in anemia prevalence seems less successful, particularly in rural area. For elderly people, the causes of anemia are much more complicated, not only just related to nutrition status, but largely relates to chronic diseases such as cancer, tumor, etc. However, considering the achievement obtained in decreasing anemia prevalence in urban middle-aged and elderly people, more than 30% reduction for those aged 60y and over, more efforts to improve nutrition status and living standards of elderly people in rural area is meaningful and is guaranteed to be successful.

(4) Vitamin A deficiency

Vitamin A deficiency is also a public health problem in developing countries. Vitamin A deficiency impairs visual function and leads to blindness if it becomes severe. Besides, vitamin A deficiency also negatively affects immune function and may be exacerbate some minerals such as iron deficiency. Massive work has been done to improve vitamin A nutrition status among infants and children while less attention for elderly population though it is important for maintaining their health.

Animal foods directly provide vitamin A while beta-carotene from plant foods can be converted to vitamin A in the body. Thus, though the animal foods consumption is limited in some rural area, the vitamin A deficiency is not severity. The nationwide vitamin A deficiency rate of elderly Chinese is 1.6% while the borderline deficiency is much higher, reach to 9.6%. The deficiency is related to economic development, more serious in rural area than that in urban (Figure 6).

Table 4 Rate of anemia for middle-aged and the elderly

		Urban			Rural		
		1992	2002	Change	1992	2002	Change
45-59y							
	Subtotal	23.2	17.6	-24.1	24	25.0	4.2
	male	16.3	13.1	-19.6	20.6	21.5	4.4
	female	29.1	21.1	-27.5	27.2	28.0	2.9
60y-							
	Subtotal	28.8	19.6	-31.9	33.5	31.6	-5.7
	male	26.2	18.3	-30.2	34.1	31.9	-6.5
	female	31.5	20.9	-33.7	32.9	31.3	-4.9

Figure 6 The rate of vitamin A deficiency and borderline deficiency in middle-aged and elderly Chinese

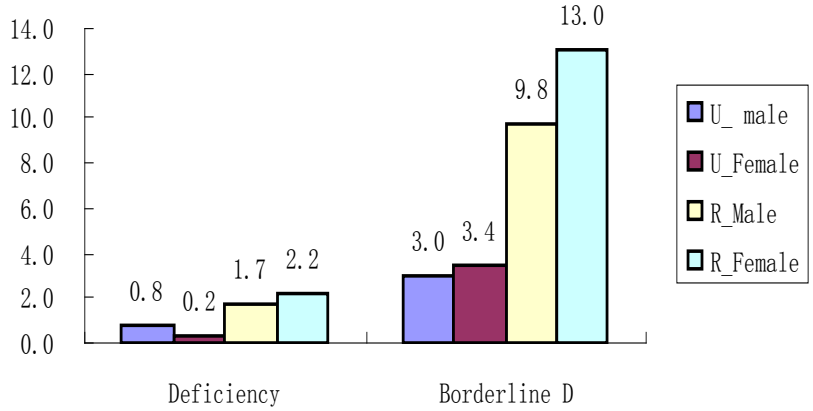


Figure 7 The rate of overweight in middle-aged and elderly Chinese

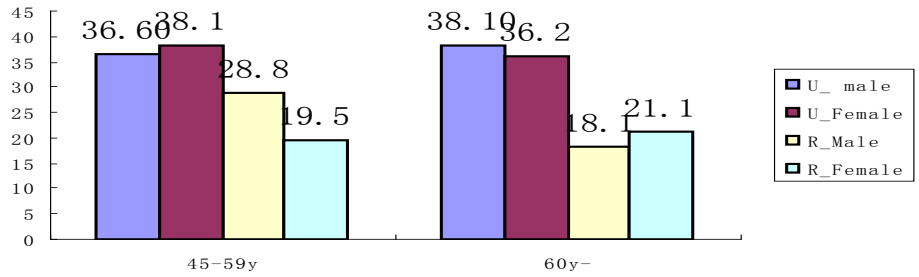
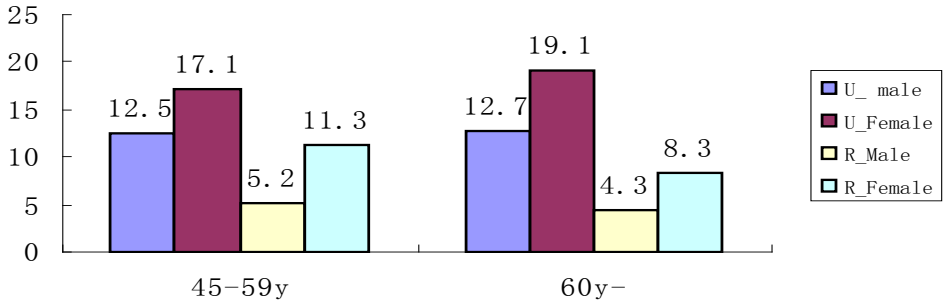


Figure 8 The rate of obesity in middle-aged and elderly Chinese



4. Over-nutrition related Diseases

(1) Over-weight and Obesity

The current changes of dietary pattern and lifestyle caused the increment of overweight and obesity. The criteria for overweight is BMI ≥24.0 and obesity is BMI≥ 28.0 in China. This criteria is different from that of USA but has its own scientific evidence for hypertension prevalence among those with BMI ≥24.0 is 2.5 times of the prevalence among people with BMI less than 24.0, and people with BMI≥ 28.0, the hypertension prevalence rate is 3.3 times of those with BMI ≥24.0. Similar situation is also for diabetes, dyslipidemia and cardiovascular disease. For middle-aged and elderly people, maintaining ideal body weight is more important to their health.

The nationwide overweight rate is 29.0% and 24.3% for middle-aged and the elderly people respectively. The overweight rate of urban people is much higher than that in rural for both age groups and only in rural middle-aged group, the overweight rate of male is obvious higher than that of female (Figure 7).

The nationwide obesity rate is 10.2% and 8.9% for middle-aged and the elderly people respectively. Similar to the status of overweight, the obesity rate of urban people is much higher than that of rural people. Different from that of overweight, the obesity rate of female is significantly higher than that of male for both middle-aged group and the elderly and urban and rural (Figure 8).

(2) Hypertension

Hypertension is one the most important risk factors for cardiovascular disease. According to previous national surveys, the prevalence and absolute numbers of hypertension have increased dramatically. The estimated number of hypertension cases among Chinese adults has increased from 59 million in 1980 to 94 million in 1990, and in 2002, this number reached 160 million. Although being treated as a focal issue and many preventive measures were taken, hypertension is still the one of the important

health problems, particularly for middle-aged and elderly people.

Data from this survey shows that the nationwide hypertension prevalence rate is 29.3% and 49.1% for middle-aged and the elderly people respectively. Very obviously, the hypertension prevalence rate is increased with age and urbanization and it seems that aging has more contribution than urbanization (Figure 9).

(3) Type 2 Diabetes Mellitus

Because of its dramatically increased prevalence, severe complications and its heavy burden on healthcare expenditure, Type 2 diabetes mellitus (T2DM) is getting more and more concerned. In China, the T2DM prevalence rate of adult was just 1% in 1980, however, this rate rapidly increased with urbanization. The nationwide T2DM rate is 4.3% and 6.8% for middle-aged and the elderly people. The T2DM prevalence is also increased with age and urbanization. Different from hypertension, the effect of urbanization seems stronger than that of aging since the T2DM prevalence of middle-aged people is nearly two times of that in rural elderly. For elderly people, female T2DM rate is higher than that of male.

Although the prevalence of T2DM among middle-aged and elderly Chinese is still lower than that of some developed countries such as United States which the rate is over 20% for elderly people, the threatening impact of T2DM becomes more and more serious. However, many studies prove that T2DM can be prevented and controlled by balanced diet, suitable physical activity and good lifestyle. The relatively lower T2DM prevalence rate among rural middle-aged and elderly Chinese also substantiates this point of view (Figure 10).

Figure 9 Hypertension prevalence in middle-aged and elderly Chinese

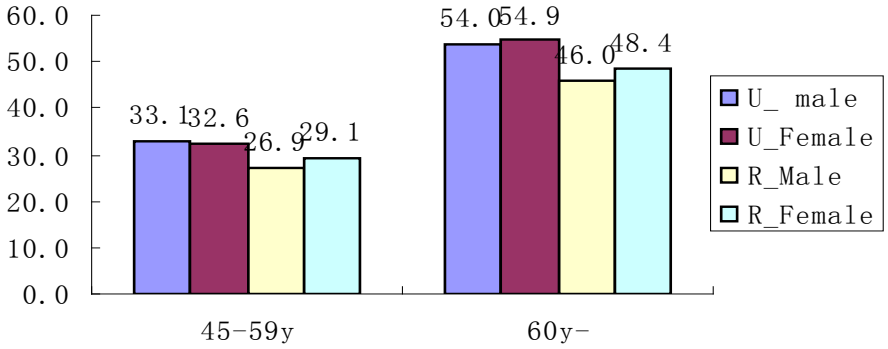
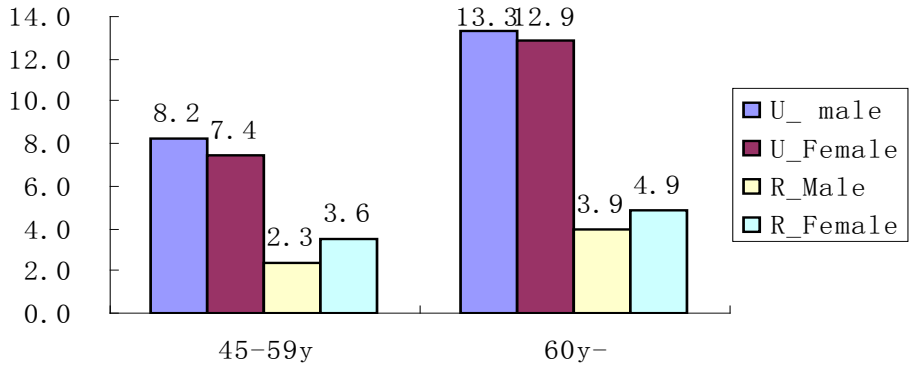


Figure 10 Type 2 Diabetes Mellitus prevalence in middle-aged and elderly Chinese



(4) Dyslipidemia

It has been proved by many epidemiological and intervention studies that blood lipid concentration is closely linked to coronary heart diseases and cerebrovascular disease. However, the national representative data about Chinese lipids level is very limited and this impeded formulation of related strategy and the prevention of dyslipidemia. The results of this survey provide national representative data on dyslipidemia.

The nationwide dyslipidemia rate is 22.9% and 23.4% for middle-aged and elderly people and the rate of urban people is higher than that of rural people. Different from T2DM, dyslipidemia rate does not increase with age but for elderly people, the rate of female is higher than that of male (Figure 11).

(5) Hypercholesterolemia

The nationwide hypercholesterolemia rate is 4.7% and 6.1% for middle-aged and elderly people and increase with age and urbanization except rural male. The hypercholesterolemia rate of female is higher than that of male both for urban and rural middle-aged and elderly people (Figure 12).

Figure 11 Dyslipidemia prevalence in middle-aged and elderly Chinese

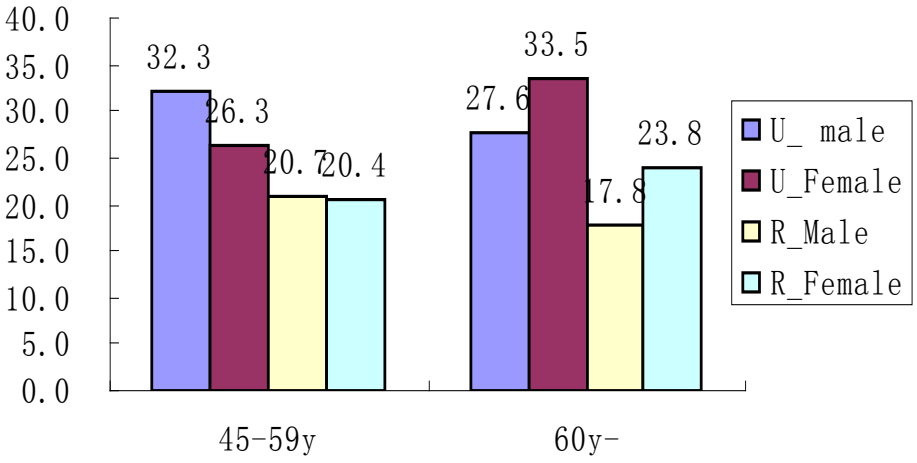
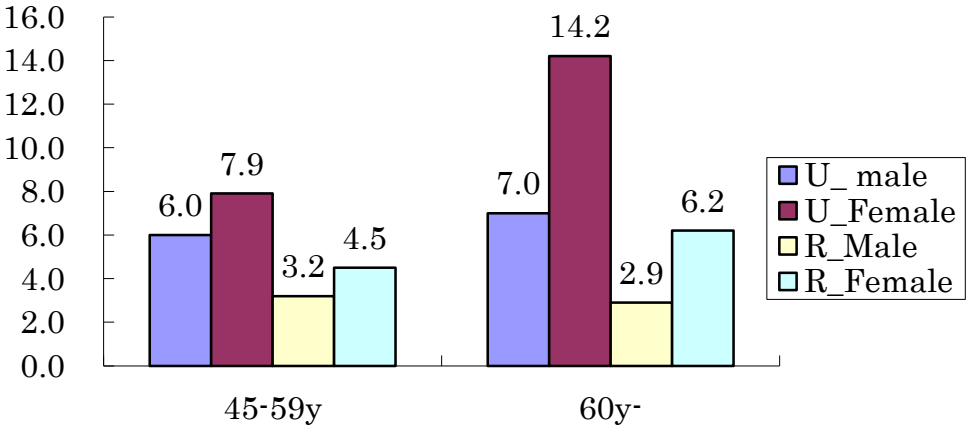


Figure 12 Hypercholesterolemia prevalence in middle-aged and elderly Chinese



(6) Hypertriglyceridemia

Triglycerides have been well established as a risk factor for coronary artery disease (CAD) for several decades. As early as 1959, higher serum triglyceride levels have been reported in patients with CAD. For a long time, triglycerides were overshadowed by other blood lipids, in particular, by low-density lipoprotein. In regard to cardiovascular disease, LDL-"bad" cholesterol was considered more important than triglycerides.

In 1994, a research group from University Of Southern California triggered a heated debate in the medical community with the publication of a study linking triglycerides to coronary artery disease, which accounts for 200,000 deaths each year. The study found that despite aggressive treatment of the bad cholesterol, patients with high triglycerides continued to suffer damage to arterial walls. Using state-of-the-art imaging techniques and specific tests, the scientists found that VLDL and IDL were the culprits.

The danger is similar to not changing the oil in a car. When neglected, both blood and oil get thick, which makes the heart or engine work harder to pump the fluid. The fluid also picks up excess debris and tends to form nasty deposits, which ultimately cause a breakdown. An engine will burn up. In humans, the end result is a heart attack or a stroke.

The problem is, people with elevated triglyceride levels almost invariably have other major risk factors for heart disease (mainly obesity, diabetes, and/or high blood pressure), and, so far, it has not been possible to sort out whether the triglycerides themselves pose an independent risk.

The nationwide hypertriglyceridemia rate is 15.7% and 14.8% for middle-aged and elderly people.

(7) Hypertriglyceridemia prevalence is more serious than hypercholesterolemia for Chinese middle-aged and elderly people, and it is the major dyslipidemia problem. The urban hypertriglyceridemia rate is higher than that in rural area. Except middle-aged urban male people, the rate of female is higher than that of male (Figure 13).

(8) Metabolism Syndrome (MS)

The metabolism syndrome (MS) is a constellation of interrelated risk factors of metabolic origin- that appear to directly promote the development of atherosclerotic cardiovascular diseases. Patients with MS also are at increased risk for developing type 2 diabetes mellitus. In the past few years, it has received increased attention in China and Chinese diabetes academic committee has set forth the MS diagnostic criteria considering the risk factors of obesity, abnormal blood pressure, glucose and dyslipidemia. However, the national representative data concerning MS prevalence in China is limited until some valuable outcomes being worked out from 2002 China Nutrition and Health Survey.

Figure 13 Hypertriglyceridemia prevalence in middle-aged and elderly Chinese

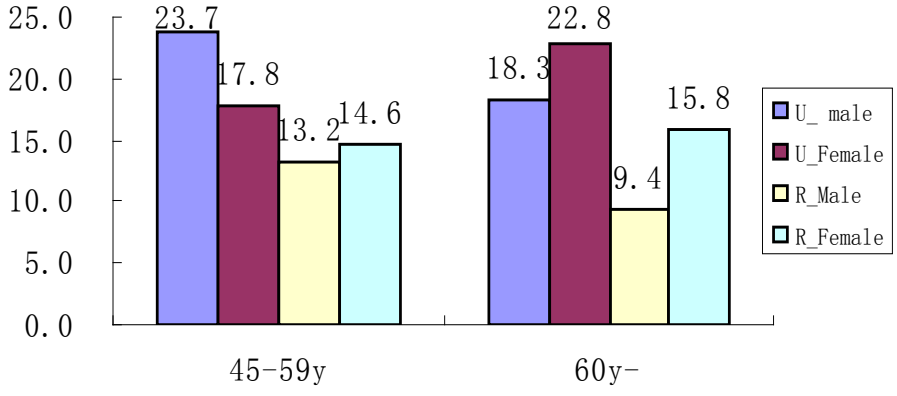
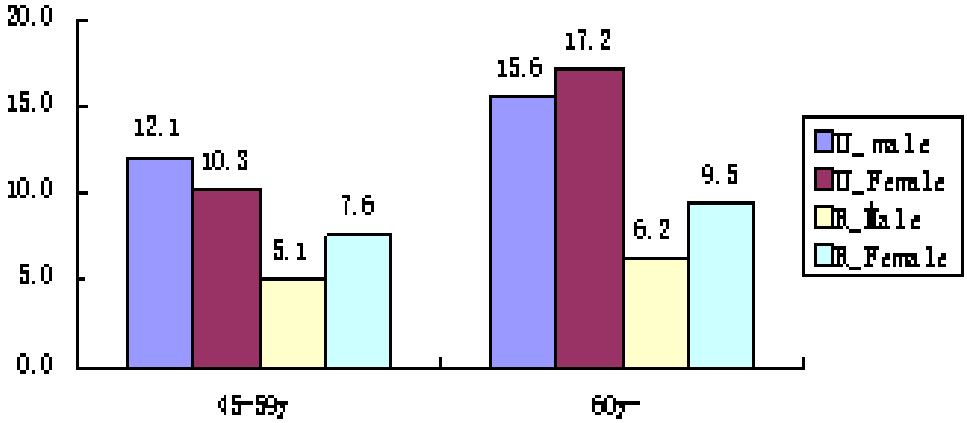


Figure 14 Prevalence of metabolism syndrome in middle-aged and elderly Chinese



The nationwide metabolism syndrome rate is 7.6% and 10.1% for middle-aged and elderly people. The rate of MS among elderly people is higher than that among middle-aged in both urban and rural area and the rate for urban people is also higher than that for rural people. Comparing to male, MS prevalence is more serious in female (Fig 14).

Discussion

The great achievement in economic not only increase the income of ordinary Chinese people but also strengthened Chinese government's ability to deal with the varieties of social problems including the medical and health care issues. Lots of programs had been and are still being implemented to fight against poverty. Children and women, particularly in poor rural area get much more concerned. The massive decrease in Chinese death rates from nutritional deficiency and infectious and parasitic diseases has been one of the most important, practical medical successes in the last decades.

However, it has to be admitted that less attention was paid for the issue of aging while the rapid demographic shift makes it more and more serious. Largely due to the one-child policy , China will take just 25 years to aging society as much as Europe did during the last century and by 2050, those older than 60 will make up 31 percent of total population , even higher than the USA. The shift of market economy has driven millions rural young people to urban area where they have more opportunities for personal development and can get more income. Thus old people in rural area have to find their own ways of support without fixed incomes and available health-care benefits are beyond reach. The problem of anemia among rural elderly population, prevalence without any reduction in recent ten years, reflect this situation. For urban elderly people, the situation seems not so serious but still face problem. The one-child policy means that one child have to be responsible for caring parents and even grandparents. This demographic trend - single child and aging parents- explains why more the preliminary schools and kindergartens are being changed intoretirement home. Actually, more and more urban aging people live alone and must take care of their life by themselves. The social, financial and physiological changes weaken foods supply for the aging people and make them at a high risk of poor nutrition and health status.

In contrast to the poor nutrition status and the communicable and infectious diseases, people with economic privilege began to suffer from a rising prevalence of chronic degenerative diseases such as obesity, cardiovascular diseases, diabetes, cancer, etc. Actually, the prevalence of these non-communicable diseases in middle-aged

population, particularly for urban people grows very fast and these diseases have a severely weakening effect on the productivity of this advantage social group. Besides, the expenditure of dealing with these 'mis-developed diseases' is costly and more public health resources are being unequally consumed by this social group already at an advantage.

Nutrition status reflects individual's life quality and the social development, and it is also the basis for eradicating communicable and preventing chronic degenerative diseases. Like other developing countries, China also faces the problem of double burden on nutrition issue, and this is particularly obvious and serious among middle-aged and elderly population. More pertinent policies for this growing people should be formulated and more basic scientific work should be done for proposing suitable nutrition advise which is respond to the changing physiological, psychological, social and economic capabilities of the elderly individuals to meet overall nutritional requirements.

Home dining over positioning of wheat food nan -From the case of Uyghur in Xinjiang China-

Mizue Kumagai*

1. Introduction

(1) The beginning

The Uyghur in Chinese Xinjiang are people who make wheat the main grain (ISHIGE・ YOSHIDA・ AKASAKA・ SASAKI 1972:156). People who make wheat the main grain do not to have a vocabulary that corresponds to syusyoku 主食 with a meaning close to staple food, and hukusyoku 副食 with a meaning close to sub-food, that composes the meaning of "meal" in a culture, the meal terminology that makes rice the main grain in Japan, Korea and China has such a vocabulary (SHINODA 1972:50, CHANG 1979 : 7-8, ISHIGE 1983:412, NAKAO 1990:84, OSADA 1995:62-63). The question is why rice is called syusyoku 主食 but wheat is not?

Why does rice correspond to syusyoku? Shinoda and Ishige indicate that rice is excellent nourishment (ISHIGE 1983:412-413, 1986:21-22, SHINODA 1972:43-59). Wheat lacks essential amino acids found in rice(YOSHIDA・ ASHIDA 1990), and for that reason, they concluded that bread is only one of a number of elements of a meal, and not a "meal" on its own. However, Shinoda reached this conclusion only by comparing the method of table setting around rice in Japan with that of bread. Ishige reached this conclusion by analyzing the effect of consumption of wheat and rice as table grains on national patterns of supply and demand of food. These researchers compared the situation of a rice-consuming country and a wheat-consuming country, and did not observe the same aspect of meaning ascribed to rice in bread for the people who eat bread daily.

The Uyghur are people who make wheat the main grain. They make a processed food item from flour, called nan, which is prepared in a process similar to bread, including making a dough leavened with yeast and baking by indirect heat. This study examined the place of Uyghur nan on the dining table and attempted to understand the meaning for them.

(2) Examination method

This research was collected from a field survey in Urumqi (乌鲁木齐 in Chinese) for approximately ten months from October 2001 to March 2002 and from May 2002 to October 2002. The investigation method was participant observation. The language used in the field was Uyghur. Chinese was also used when the situation required it.
Uyghur is written using Arabic script in present Xinjiang. However the Uyghur characters in this thesis use the UKY, method for writing Uyghur on the computer.

(3) Outline of investigation region

1) Urumqi

* Visiting Researcher, National Museum of Ethnology, Osaka, Japan

Urumqi is the district capital of Xinjiang Uyghur Autonomous Region. The population of Urumqi is 1,815,296; Uyghur are 13% of the population (241,008), and Han are 73% of the population (1,327,454) (Statistic Bureau of Xinjiang Uyghur Autonomous Region 2004).

Before the liberation, most all homes were one-story high (Urumqi City Local Record Compilation Committee Editorial Office 1987:23) and the population was about 100,000 people, and it was present 1/18. After the liberation, the population increased rapidly through migration. Present Urumqi is a city lined with the skyscrapers that are ten-stories high. The diffusion rate of the household appliance such as televisions, refrigerators, and washing machines are now more than 100 %(Urumqi History of a Party Local Record Compilation Committee 1994:252).

2) Surveyed homes

This research was conducted in three homes. The three homes, occupations, residences, and hometowns are different from each other. This research tries to show characteristics by finding common qualities in the three homes, and trying to illustrate the current state of Urumqi from it. To show these homes easily, call the homes as home-A, - B, -C. People in the homes will be described as follows. The capital letter shows the home name and the lower case letter shows the location in the family structure. The father is written in “f”, the mother is “m”, the son is “s”, the daughter is “d”, and the mother’s mother is “mm.” For example, the father in home-A will be shown as Af. The second daughter in home-C will be shown as Cd2.

Next, the people in the homes, their occupations, and the individual home will be described. Five people live in Home-A: the father, mother, oldest daughter, oldest son, and second daughter. Af was born in Ghulja (伊宁) and came to Urumqi with his parents when he was a year old. Am was born in Urumqi. She is the only person who was born in Urumqi among the parents in the three homes. Af’s occupation is to help his friend’s business in such ways as driving a motorcycle. His income is about 1000 to 2000 yuen a month. Am helps her friend's business. Her income is about 600 yuen a month. Ad1 is a university student. As1 is a high school student. Ad2 is a junior high school student.

Four people live in Home-B: father, mother, oldest daughter, and second daughter. In addition, the maternal mother, Bmm, who lives close to home-B frequently visits home-B. Bf came from Aqsu (阿克苏) when he began college. Bm came from Chöchek(塔城)because of her parent's work when she was a year old. Bf is a teacher at the university. His income is about 3000 yuen. Bm is a full-time housewife. Bd1 is a university student. Bd2 is a high school student.

Five people live in Home-C: father, mother, oldest daughter, second daughter, and maternal mother. Cf comes from Qeshqer (喀什) and Cm comes from Yeken (莎车). They both came to Urumqi when they started high school. Cf is a merchant; the amount of his income is uncertain. Cm works in the service industry at the university. Her income is about 800yuen a month. Cd1 and Cd2 are junior high school students. Cmm is unemployed. Her days are spent cooking and she goes to see a number of her friends at their homes.

(4) Uyghur meals

1) Uyghur dishes

This paragraph introduces Uyghur dishes and discusses the daily table conditions of the home. Meals in Uyghur homes start with *nan*. *Nan* is food with a long history in Uyghur. In the book that introduces Uyghur folk culture, ABUDUKÉRIM describes *nan*：“It is a basic, traditional food, which is the basis of the daily meal. First of all, *nan* will not be lost, and Uyghur daily live primarily takes place in the home. (ABUDUKÉRIM 1996：31).”

Some other flour processed foods are eaten in addition to *nan*. *Quymaq* is deep-fried bread. *Sangza* is thin fried cakes. *Baqali* is pound cake. *Pichine* is cookies. These flour foods might add *nan* to the ending of a word, such as *pichine-nan* and *baqali-nan*. The usage of the word shows the concept of *nan* widely comprises flour processed food in Uyghur. This word is also used for *mómó* (Chinese) for Han food called *höl- nan* in Uyghur. *Höl* means "damp."

Tea is served with such flour-based food. The Uyghur word for tea is *chay*. After being baked, *nan* quickly becomes hard. Water to soak *nan* and make it soft is indispensable. *Chay* is an essential beverage for eating *nan*. There are other drinks besides *chay*. *Qétiq* is yogurt. *Halwa* is a hot drink made from sheep fat, flour, and sugar water. Fruits with a lot of moisture, such as watermelons, melons, or grapes are also served with *nan*. Moreover *chöchüre* (won ton soup), *ügre* (noodles), *showgürüch* (rice porridge), and *suyuqash* (soup with pieces of boiled flour) are also served with *nan*. Though the name *suyuqash* is assumed to be only for boiled flour at a restaurant in Urumqi, the original meaning of *suyuqash* is "suyuq (watery)" and "ash (dish)", and the two words are an inclusive concept for all soup dishes.

Besides these, there are *manta* and *raghmen* and *polu* served as a dish without soup. *Manta* is a steamed meat bun. *Raghmen* is boiled noodles covered with stir-fried meat and vegetables. *Polu* is rice cooked with carrots and lamb. These dishes are called *qoyuq ash* (thick dishes) as compared with *suyuq ash*. Because these dishes do not have soup compared with the *suyuq ash*, they are not put on the table with *nan*.

From such *suyuq ash* to *qoyuq ash*, the dishes of Uyghur are cooked with one kind of main dish, one fire and one pan. It is rare that two or more dishes are combined at one meal time.

2) Aspect of table in the home

The Uyghur table has a peculiarity that depends on nan always being on the table. Because of a dry climate, *nan* never spoils in Xinjiang. Therefore, *nan* needs neither cooking before it is eaten nor preservation in the refrigerator. That is, there is no necessity for putting *nan* on the table because it is always left on the table. Each person can eat however much *nan* is desired in each meal

Photograph 1 *Nan:* left on the table. It's able to eat anytime.

In general, the amounts of *nan* put on the table is three to four piled up pieces looking like a mountain. The mountain of *nan* is replenished on the table as necessary. Ishige classifies preparing a table into two methods (ISHIGE 1991). One is *ku-kan tenkai gata* (空間展開型, Japanese) which is the method of setting all dishes on a table at one time. Meals in Japan, Korea, China and India correspond to *ku-kan tenkai gata*. Another method is *jikeiretu gata* (時系列型, Japanese) which is the method of serving dishes one by one in a specific sequence. European meals served in courses correspond to this method. However, the case observed in the Uyghur home is that dining does not fit either method. I would like to add a new category, *ho-chi gata* (放置型, Japanese, leaving type), to have three eating methods.

2. Concept of meal of Uyghur meal with nan

(1) Nan is food that does not "eat"

This paragraph analyzes the meaning of the verbs "eat" and "drink" in Uyghur. It often happens that "eat" and "drink" might

indicate a different meaning in a different culture.

The act of drinking is important in Urumqi. The reason is that water is indispensable in the dry climate. People eat melon and watermelon and drink tea frequently especially in summer and eat *nan* when they drink tea. People express about this like "When drinking tea, it is natural to have *nan*." According to this saying, it is possible to say that drinking tea and eating *nan* are concerted actions.

To drink something with *nan* is said to"*chay itish* (drink tea)" in Uyghur. The phrase "eat *nan*" is not used. "Drink tea" is expressed with "eat *nan*" in mind. When drinking tea without *nan*, the emphasis is needed such as "*sin chay itiish* (drink clear tea)." The word "meal" in Uyghur is "*tamaq*" and the verb corresponding to "*tamaq"* is only "*yeish* (eat)."

For the Uyghur who living in a dry region, *nan* is a food always left on the table. *Nan* can be eaten at any time, and need not be finished at one meal time. As a result, the word "meal" in Uyghur is recognized as a situation distinguished from "eat *nan*." Through the following cases, I try to explain this perspective from the situation seen through eating at home. Table 1 shows the reference to the meal and the meal number in the following cases.

Case1: Home-A, June 22, Time 21:30
Conversation about meal number A-7
Investigator "After returning home, did you **eat** any **meal** (***tamaq***) today? (*bügün öyge qaytqandin keyin siz tamaq yedingizmu?*)"
Am "No, I did not **eat anything** at all. (*yaq,men hich nimé yeymidim*)"
Ad1 "Mother, did you **drink tea**?" (*apa, nanchay éshtingizmu?*)
Am "Yes, I **drank**." (*hee,éshtim*.)

Case 2: Home-A, June 25, Time 17:00
Conversation before meal number A-43
Am "Did you **eat** a **meal**(***tamaq***) today?(*bügün siz tamaq yedingizmu*?)"
Investigator "I **ate** *nan* and rice porridge in the morning with Ad1, and in the day time, I **drunk** rice porridge that As1 made." (*etigende **Ad1** bilen shipen ,nan yedim*. *chüshte **As1** étdgan shipen eshtim*.)
Am "Then, you have not **eaten anything** yet today." (*undaqta siz hitch nimé yimidingizgu*.)

Am who understand that I ate nothing, gave *polu* after this conversation, Am, Ad2, and me ate it.

Case 1 shows the people treating "drinking tea (with eat *nan*)" besides the word "meal (*tamaq*)." Case 2 shows people treating not only the act of drinking tea (eat *nan*) but also to eat dishes such as rice porridge besides the word "meal (*tamaq)*" and "eat."

From Case 1 and Case 2, it is possible to find clear distinction for drinking tea and rice porridge from "eat" and "meal (*tamaq).*"

What is *tamaq* in Uyghur? The following paragraph analyzes the meaning of it.

(2) Tamaq is the one eat alone

In this paragraph, I try to classify the one that *tamaq* and is not *tamaq* with a number of indexes. Table 2 shows the classification process in this paragraph. The item of the classification is made from referring to the menus from Urumqi restaurants and the item written in the ABUDUKÉRIM's ethnography.

The division with that which is *tamaq* and that which is not *tamaq* is lucidly answered when asking people. People answer "What is cooked warm and has meat or flour is *tamaq.*" This answer does not correspond to the attitude that makes rice porridge outside *tamaq*. What is cooked as it is warm is called "*tamaq,*" and other cakes, juices, tea, and *nan* are not called *tamaq* (KUMAGAI 2004). This is assumed to be the first stage of the classification (Table 2· classification 1).

Table 1 Table observation data in Home-A, B, C

Table observation record of home-A

June 22(Sat)

Meal #	Time	Type	Eaten content	Af	Am	Ad1	As1	Ad2	Inv	Neices of Am
A-1	7:00	○	nan· milk tea	T1						
A-2	8:30	○	nan· milk tea		T1		T1	T1		
A-3	12:30	○	nan· tea		T1				T1	
A-4	14:30	▲	gengpen(rice)			T1	T1	T1	T1	T1
A-5	16:00	○	apricot			T1	T1	T1	T1	T1
A-6	18:00	○	apricot		T1	T1	T1	T1	T1	T1
A-7	19:30	○	nan· tea		T1		T1			
A-8	20:30	○	nan· tea	T1						
A-9	21:30	○	nan· tea· watermelon	out side			out side			
A-10	24:00	○	nan· tea			T1			T1	

June 23(Sun)

Meal #	Time	Type	Eaten content	Af	Am	Ad1	As1	Ad2	Inv	Neices of Am
A-11	7:00	○	nan· soup	T1						
A-12	8:00	○	nan· soup		T1			T1		
A-13	8:30	○	nan· soup				T1			
A-14	9:00	○	nan· soup			T1		T1	T1	
A-15	11:30	○	nan· tea		no seat					
A-16	13:00	▲	guà-miàn(noodle)		T1	T1	T1	T1	T1	
A-17	13:30	○	nan· tea		no seat					
A-18	15:30	○	nan· tea· salad					T1		
A-19	16:00	○	nan· tea· salad			T1		T1	T1	
A-20	16:30	○	apricot			T1	T1	T1	T1	
A-21	19:00	○	watermelon		T1	T1	T1	T1	T1	T1
A-22	21:00	▲	polu(rice)		T1			T1	T1	
A-23	21:30	▲	polu(rice)	T2			T1			
A-24	22:00	▲	polu(rice)			T1				T1

June 24(Mon)

Meal #	Time	Type	Eaten content	Af	Am	Ad1	As1	Ad2	Inv	Guest
A-25	6:00	○	nan· tea		T1					
A-26	7:30	○	nan· milk tea	T1			T1	T1		
A-27	8:30	○	nan· milk tea			T1				
A-28	9:00	○	nan· tea	T1			T1			T1
A-29	11:30	○	nan· milk tea					T1	T1	
A-30	13:00	▲	polu(rice)(Ad2nan)				T1	T1	T1	
A-31	13:30	○	apricot				T1	T1	T1	
A-32	15:30	○	watermelon			T1	T1	T1	T1	
A-33	16:30	○	nan· tea			T1	T1	T1	T1	
A-34	19:30	○	nan· tea· sugar	T1						
A-35	21:30	□	nan· suyuqash(soup)	T1	T1		T1	T1	T1	
A-36	23:00	○	nan· tea			T1			T1	

June 25(Tue)

Meal #	Time	Type	Eaten content	Af	Am	Ad1	As1	Ad2	Inv
A-37	7:00	○	nan· porridge		T1			T1	
A-38	7:30	○	nan· porridge	Kitchen			Kitchen		
A-39	8:00	○	nan· porridge			T1			T1
A-40	11:30	○	apricot				T1		T1
A-41	12:30	○	nan· porridge				T1		T1
A-42	16:30	○	apricot· cookie					T1	T1
A-43	18:00	▲	polu(rice)		T1			T1	T1
A-44	19:00	▲	polu(rice)				T1		
A-45	20:30	△	juwawa(boiled dumpling)	T1	T1	T1	T1	T1	
A-46	22:00	○	nan· tea			T1			T1

Table observation record of home-B

September 3(Tue)

Meal #	Time	Type	Eaten content	Bf	Bm	Bd1	Bd2	Bmr	Inv	Neighbors		Friends of Bd1		Daughters	
B-57	6:30	○	nan· milk tea· dry fruits	T3	T3		T3	T3							
B-58	8:00	○	nan· milk tea· dry fruits		T3	T3			T3						
B-59	12:30	△	manta· milk tea· dry fruits		T3	T3		T3	T3						
B-60	15:00	○	nan· tea		road			road	road	road					
B-61	16:00	△	manta· milk tea· dry fruits		T3			T3	T3						
B-62	17:00	△	manta· milk tea· dry fruits			T3						T3			
B-63	18:30	○	peach· tea		T5	T5		T5	T5						
B-64	19:30	△	manta· tea											T5	
B-65	20:30	△	manta· tea	T5											
B-66	21:00	△	manta· tea· dry fruits	T3	T3	T3	T3	T3	T3						

Table observation record of home-C

July 14(Mon)

Meal #	Time	Type	Eaten content	Cf	Cm	Cd1	Cd2	Cmr	Inv	Relative		Driver		Relative	
C-72	6:00	○	nan· tea					T2							
C-73	7:00	△	nan· tea· sey	T3	T3	T3	T5	T5	T5	T3				T3	
C-74	10:00	△	nan· tea· sey							T3		T3			
C-75	10:30	△	nan· qordaq(fried dishes)	T1	T1	T3	T3	T3	T1	T1		T1		T3	
C-76	11:30	○	melon	T3	T3	T3	T3	T3	T3					T3	
C-77	12:00	△	nan· qordaq(fried dishes)	T3	T3	T3	T3	T3	T3					T3	
C-78	17:30	○	melon			no seat									
C-79	18:00	○	peach	no seat											
C-80	18:30	–	ice cream				no seat								
C-81	21:00	□	nan· suyuqash(soup)	T2	T2	T2	T2	T2	T2						

July 15(Tue)

Meal #	Time	Type	Eaten content	Cf	Cm	Cd1	Cd2	Cmr	Inv	Relative		Driver	
C-82	7:30	○	nan· tea· sey	T2	T2	T2	T2	T2	T2				
C-83	12:00	△	juwawa(boiled dumpling)		T2								
C-84	12:30	△	juwawa(boiled dumpling)			T2	T2		T2				
C-85	13:00	○	nan· tea· dry fruits		T1							T1	
C-86	13:30	△	juwawa(boiled dumpling)			T2	T2		T2	T2			
C-87	14:00	△	juwawa(boiled dumpling)					T2					
C-88	14:30	○	nan· tea· dry fruits				T2		T2	T2			
C-89	16:00	△	juwawa(boiled dumpling)	T2			T2		T2	T2			
C-90	16:30	○	melon				T3		T3	T3			
C-91	17:30	○	melon			no seat							
C-92	19:00	□	nan· suyuqash(soup)	T2	T2	T2	T3	T2	T3				

July 16(Wed)

Meal #	Time	Type	Eaten content	Cf	Cm	Cd1	Cd2	Cmr	Inv	Friends of Cd2		Two guests		Relative	
C-93	7:00	○	nan· tea					T2							
C-94	9:30	○	nan· tea					T2	T2						
C-95	10:30	○	nan· tea· dry fruits				T2		T2	T2					
C-96	13:00	○	nan· tea· dry fruits	T1	T1	T1	T1		T1			T1			
C-97	13:30	▲	lengmen(boiled noodle)	T1	T1	T3	T1	T3	T1			T2			
C-98	14:00	○	lengmen(leftover)				no seat								
C-99	15:00	○	melon			no seat									
C-100	16:00	○	melon	T3		T3	T3		T3						
C-101	18:00	○	nan· tea			T3									
C-102	19:00	○	nan· tea			T3									
C-103	20:00	□	suyuqash(soup)		T3										
C-104	21:00	○	nan· tea· dry fruits	T2		T2	T2		T 2						
C-105	21:30	○	nan· tea· dry fruits	T2	T2	T2	T2		T 2					T 2	

Meal sign explanatory notes

○ "chay" (nan· tea· fruit· dry fruit)
□ "tamaq" with soup
△ "tamaq" changes into "chay"
▲ "tamaq"

Action sign explanatory notes

meal (The character indicate a place where meal was taken.)
go out
sleep

※「Inv」is「Investigator」

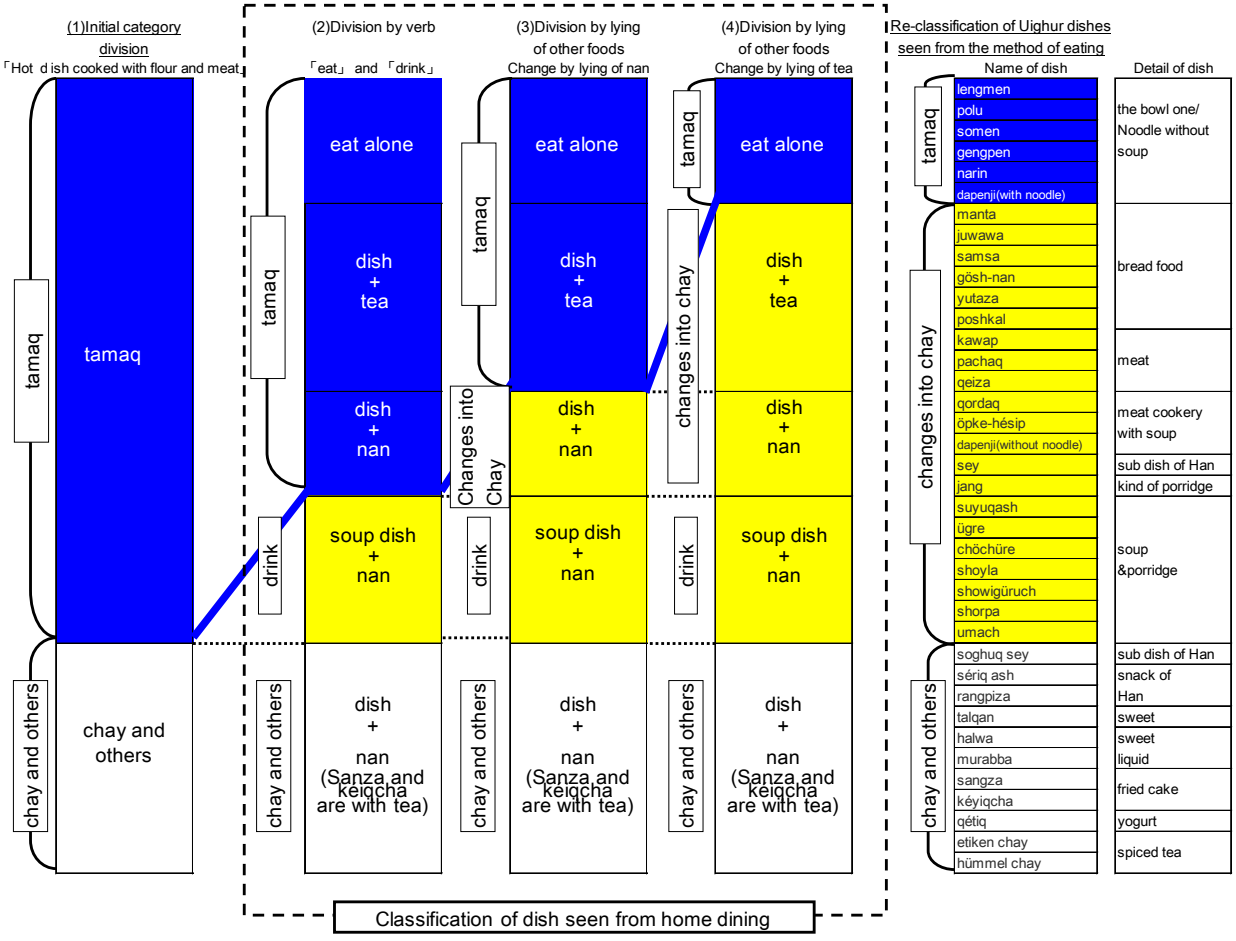
※Time was delimited every 30 minutes.
※The analysis of the meal place is omitted in this thesis.

The second stage was classified from the verbs "eat" and "drink"(Table 2: classification 2). Soup is taken with the verb "drink" and that is taken with *nan*. This was described in Chapter 1, paragraph 4.1, and Chapter 2, paragraph 1, Case 2. Uyghur soup is eaten with the verb "drink" and is not considered to be "eat" "*tamaq*." These dishes can be considered to be an intermediate category between *tamaq* and not *tamaq*.

The classification of the third and fourth stages made from an opportunity that changes *tamaq* into *chay* by the way of eating (Table 2: classification 3, 4). This stage is made from the case that *manta* which was one of *tamaq* was eaten using the vocabulary "Let's drink tea" (Table 1: meal number B59-66). Why is *manta* that must be corresponding to *tamaq* called *chay*? When I asked this question of Ad1 and Bd1, they answered as follows: "If the eaten amount is little, *tamaq* is called *chay* (Ad1)," and "When tea comes out and it is soaked in it, it is *chay* (Bd1)." That is, *tamaq* is changed into "*chay* (not meal)" when it is possible to be eaten by soaking in tea or when it is possible to add and subtract the amount eaten.

The appearance of division between *tamaq* and *chay* changes the form when paying attention to the method of eating. From the results of this classification, the conclusion can be that the dishes with the soaked element and adjustability of amount are changed into *chay*, and the *tamaq* which does not change to *chay* are the dishes eaten alone and without soup and distributes a plate individually. A phased classification in Table 2 shows the changes that for the one that was *tamaq*, most changes into *chay* when the observation of the eating method is added. Most Uyghur *tamaq* are *chay* when seen from the method of eating at home.

Table 2 Re-classification of Uighur dishes seen from home dining



(3) Meal during the day through combining tamaq and chay

Meals based on this division are not in the form of morning-meal, noon-meal, or evening-meal as *asa-gohan* (朝ごはん), *hiru-gohan* (昼ごはん), *ban-gohan* (晩ごはん) in Japan or *zǎo-fàn* (早饭), *wǔ-fàn* (午饭), *wǎn-fàn* (晚饭) in China. If the content of the meal during a day is seen, the meal comes out like the "type" classification in Table 1, a few *tamaq*, and the frequent *chay*. If it is right to think of *tamaq* as the "meals" in Japan or China (*gohan* or *fàn*), Table 1 shows very few in any day at any home. *Tamaq* is once in one day or sometimes not eaten in a day also happens. This means only a few *tamaq* are eaten in a Uyghur home. *Chay* is eaten six times a day and as many as seven times depending on people's situation. The frequency of this *chay* shows the existence of the different combination categories of Uyghur meals. The meals of Uyghur are composed mainly of *chay*, which is combined with *nan* which is left on the table all day long. This is a meal culture that places *nan* at the center of the meal.

(4) A characteristic of meal acts around *nan*

Most of meal acts of the Uyghur are *chay* that is doing around the food *nan* which is left on the table and edible anytime. This meal condition did not produce the form that a family eats alone each. The situation that a meal is possible to eat anytime, and the quantity can modify freely; the characteristic of this conditions promoted adversely is posture to share a meal (Table 3). I will read the example of share

Case3: Home-A, June 24, Time 15:20

Conversation about meal number A-32, 33

Ad1:"Because I ate only one bread of the onion at school today, I am hungry."

Ad1 back from a school to home at 3:20 and ate a watermelon with As1, Ad2, me who were in the home-A (A-32). Afterward, Ad1 pours the tea for four (Ad1, As1, Ad2, me) with the words mentioned above at 4:30,four people surrounded the *nan*, and drank tea (A-33). As1, Ad2 ate only one fragment of *nan*, and Ad1 ate around half of the *nan* (approximately 15cm in diameter) in the others.

The Case shows the existence of one recognition that when an individual is hungry, members at home are considered to share the place together. Ad1 set up the table by the sense of hunger of own. As1 and Ad2 just finished a meal at the noon, but they were not going to avoid eating its meal. And there were not the words that the Ad1 was anxious about they were full or not. And by this meal, Ad1 whom they told to be hungry ate rather much *nan* than the person at home, and other members ate a small piece of *nan*. In other words, in this dining table, one does not need to eat other than a small piece of *nan*, and they can only to drink tea if not hungry. It may be said that two people who were in the home did not eat by a reason to be hungry. They, so to speak, ate simply because they were present there.

Next, I examine the example of the dining table with the person who is not a resident.

Case4: Home-C, July 14, Time 7:30

Conversation about meal number C-74,75

Five residents, I, three person of the relatives of home-C, nine people in total had breakfast with a *sey* (a fry-up with much soup) and *nan* and tea.

at 9:50, all the members almost stopped eating,

Cm say that "Let's cook *qordaq* (a traditional fry-up with much soup) "

When breakfast was just over, it was said. And *qordaq* was the dishes to cook for the driver of the car that two relatives returned home from now on. 2 drivers come at 10:05 and at first are met in *nan*, tea, *sey* (meal number C-74) and the place was offered to only the 2 drivers. The residents cooked in all the members. Cf and relative (an adult male) sometimes sit down and talk with the 2 drivers.

Table 3 The rate of agreement of the meal participation number of people

Home-A
June 22(Sat)

	Eaten content	Number of people in the house	Number of people at the dining table	Numerical rate of agreement	state
1	nan・milk tea	1	1		
2	nan・milk tea	3	3	100%	
3	nan・tea	3	2	66%	*Ad1 absence
4	gengpen(rice)	5	5	100%	
5	apricot	5	5	100%	
6	apricot	6	6	100%	
7	nan・tea	2	2	100%	
8	nan・tea	3	1	33%	A f eats individually
9	nan・tea・watermelon	6	2*		
10	nan・tea	2	2	100%	

The rate of agreement of the number of people of June 22
90.00%

The rate of agreement of the number of people = in the house+on the dining table
The example of numerical value 1/1 is excluded
Home-A = Five residents + author
9 was excluded from a rate of agreement because the food was carrying out from the home.
*3, Ad1 was cooking.

Home-A
June 23(Sun)

	Eaten content	Number of people in the house	Number of people at the dining table	Numerical rate of agreement	state
11	nan・soup	1	1		
12	nan・soup	2	2	100%	
13	nan・soup	2	1	50%	As1 eats individually
14	nan・soup	3	3	100%	
15	nan・tea	4	1	25%	Am eats individually*
16	guà-miàn(noodle)	5	5	100%	
17	nan・tea	4	1	25%	Am eats individually*
18	nan・tea・salad	1	1		
19	nan・tea・salad	3	3	100%	
20	apricot	4	4	100%	
21	watermelon	6	6	100%	
22	polu(rice)	3	3	100%	
23	polu(rice)	4	2	50%	tamaq refusal
24	polu(rice)	6	2	33%	tamaq refusal

The rate of agreement of the number of people of June 23
68.75%

*15, 17 Am eat nan while walking.

Home-A
June 24(Mon)

	Eaten content	Number of people in the house	Number of people at the dining table	Numerical rate of agreement	state
25	nan・tea	1	1		
26	nan・milk tea	3	3	100%	
27	nan・milk tea	1	1		
28	nan・tea	4	3	75%	reception of the visitor
29	nan・milk tea	2	2	100%	
30	polu(rice)(Ad2nan)	3	3	100%	
31	apricot	3	3	100%	
32	watermelon	4	4	100%	
33	nan・tea	4	4	100%	
34	nan・tea・sugar	5	1	20%	A f eatl individually
35	nan・suyuqash(soup)	5	5	100%	
36	nan・tea	2	2	100%	

The rate of agreement of the number of people of June 24
85.71%

Home-A
June 25(Tue)

	Eaten content	Number of people in the house	Number of people at the dining table	Numerical rate of agreement	state
37	nan・porridge	2	2	100%	
38	nan・porridge	2	2	100%	
39	nan・porridge	2	2	100%	
40	apricot	2	2	100%	
41	nan・porridge	2	2	100%	
42	apricot・cookie	2	2	100%	
43	polu(rice)	3	3	100%	
44	polu(rice)	3	1	33%	tamaq refusal
45	juwawa(boiled dumpling)	5	5	100%	
46	nan・tea	2	2	100%	

The rate of agreement of the number of people of June 25
92%

Home-B
September 3(Tue)

	Eaten content	Number of people in the house	Number of people at the dining table	Numerical rate of agreement	state
57	nan・milk tea・dry fruits	3	3	100%	
58	nan・milk tea・dry fruits	3	3	100%	
59	manta・milk tea・dry fruits	4	4	100%	
60	nan・tea	11	10	90%	*B d1absence
61	manta・milk tea・dry fruits	4	4	100%	
62	manta・milk tea・dry fruits	5	2	40%	reception of the visitor
63	peach・tea	4	4	100%	
64	manta・tea	3	1	33%	visitor eats individually
65	manta・tea	3	1	33%	B f eats individually
66	manta・tea・dry fruits	6	6	100%	

The rate of agreement of the number of people of September 3
82.60%

*60, Bd1 was talking on the telephone in a room.

Home-C
July 14(Mon)

	Eaten content	Number of people in the house	Number of people at the dining table	Numerical rate of agreement	state
72	nan・tea	1	1		
73	nan・tea・sey	9	9	100%	
74	nan・tea・sey	11	4	36%	reception of the visitor
75	nan・qordaq(fried dishes)	11	11	100%	
76	melon	7	7	100%	
77	nan・qordaq(fried dishes)	7	7	100%	
78	melon	5	1	16%	Cd1 eats individually
79	peach	5	1	16%	Cf eats individually
80	ice cream	3	1	33%	Cd2 eats individually
81	nan・suyuqash(soup)	6	6	100%	

The rate of agreement of the number of people of July 14
72.30%

Home-C = Five residents + author

Home-C
July 15(Tue)

	Eaten content	Number of people in the house	Number of people at the dining table	Numerical rate of agreement	state
82	nan・tea・sey	6	6	100%	
83	juwawa(boiled dumpling)	5	1	20%	Cm eatl individually
84	juwawa(boiled dumpling)	4	3	75%	Cmm absence
85	nan・tea・dry fruits	8	2	25%	reception of the visitor
86	juwawa(boiled dumpling)	6 (8)	5 (7)	83.33%	Cmm absence
87	juwawa(boiled dumpling)	6 (8)	1 (3)	37.50%	Cmm eatl individually
88	nan・tea・dry fruits	5	5	100%	
89	juwawa(boiled dumpling)	6	6	100%	
90	melon	5	5	100%	
91	melon	5	1	20%	Cd1 eatl individually
92	nan・suyuqash(soup)	6	6	100%	

The rate of agreement of the number of people of July 15
66.12%

*At the time of table 86 and 87,table 85 continues receiving a visitor
Numerical rate of agreement calculated excluding the visitor.

Home-C
July 16(Wed)

	Eaten content	Number of people in the house	Number of people at the dining table	Numerical rate of agreement	state
93	nan・tea	2	2	100%	
94	nan・tea	2	2	100%	
95	nan・tea・dry fruits	5	4	80%	Cmm absence
96	nan・tea・dry fruits	8	7	88%	Cmm absence
97	lengmen(boiled noodle)	8	8	100%	
98	lengmen(leftover)	4	1	25%	tamaq refusal
99	melon	3	1	33%	Cd1 eatl individually
100	melon	4	4	100%	
101	nan・tea	1	1		
102	nan・tea	1	1		
103	suyuqash(soup)	5	1	20%	Cm eatl individually
104	nan・tea・dry fruits	4	4	100%	
105	nan・tea・dry fruits	6	6	100%	

The rate of agreement of the number of people of July 16
76.47%

Note1: "state" explains the situation that was not seen of the agreement.
Note2: "tamaq refusal"and "reception of the visitor" was the situation that the nonparticipation is seen in conventionally.

At 10:55, the cooking of the *qoudaq* is over. 2 drivers, relatives, Cf, Cm,I (I was treated like a visitor), 6 people in total ate *qordaq* and *nan* at the table of the parlor and other residents also ate a *qoudaq* (and *nan*) at the small table of the living room (meal number C-75).

In the example mentioned above, residents did not stop other meals (here, *nan* tea, *sey* of the morning) even if there is the meal to cook just after that. They ate *nan* and tea of the early morning and start cooking. The time lag with these meals was only one hour. However, both meals are the dishes that the addition and subtraction are possible in individuals. As if it showed the point, the second meal the residents who had breakfast and unnecessary to joining the dining table also eats a *qoudaq* with the other table.

Dishes in here are both possible to decide quantity freely, and do not need to finish at all. The left *qoudaq* at C-75 is eaten once again at noon.

This two meal conditions accept eating again and again. And at the place of such a meal, a question to consider whether the people whom there is there are hunger or are not hungry is not given off in the same way as Case3 at home-A.

3. Conclusion

This research has aimed to clarify the composition of the dish in a culture that makes flour the main grain. The Uyghur meal is made by two forms. The first one is a "*chay*" the combination of *nan* and liquid, the second is "*tamaq,*" which corresponds to "meal," and is a dish without the soup and eaten alone. This is a different cultural frame from that of the concept of *syusyoku*, meaning almost staple food, and *hukusyoku*, meaning almost sub-food, that composes the meaning of "meal" in a culture that centers on rice. Such meals don't take a form to eat meals three times on a day like a *gohan* in Japan, but with consecutive "*chay*" that is shared between people who were there. This dining table invites peoples anytime, and people possible to share the place anytime. What kind of social meaning does this place take on among the lives of the Uyghur? To answer the question, the investigation that paid more attention to the social activity outside the home is necessary.

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Chapter 4.

The Role of Physical Activity and Nutrition

Can we establish the Dietary Reference Intakes for Japanese elderly?

Satoshi Sasaki*

Can we establish estimated energy requirement in the Dietary Reference Intakes for Japanese elderly? It is a great task because of the essentiality of energy on health management by nutrition throughout the life. Requirement of energy intake is defined as energy needs to keep body weight. But the reliable measurement method is not yet established.

Measurement of energy expenditure measured with doubly water technique is broadly used with consideration of weight change during the measurement period. This method has strength of relatively light physical and mental burdens of subjects, while it has a weakness that the measurement is expensive and requires special technique, and in addition, that physical activity level should be measured at same time to the same subjects. The fully-reliable measurement method is not yet established for physical activity. At the present time, either physical activity record or three-dimensional accelerometer seems usually to be used for this purpose.

True energy requirement varies at individual level. One American study reported that the standard deviation was about 200 and 160 kcal/day, in adult (excluding elderly) men and women, respectively. Therefore, a large-scale epidemiologic study with highly standardized measurements is required for the establishment of the values used in dietary guidelines such as the Dietary Reference Intakes. In order to accomplish this task, the highly-scientific epidemiological technique for study design and the ability to perform it is important.

To date, only one study had a reliable science level using national representative samples, which was usable for the development of the Dietary Reference Intakes for Japanese (2010). The subjects were however restricted to middle-aged men and women, and. it measured physical activity very roughly. No study with this level does exist for elderly Japanese. Because of scarcity of reliable data, the Dietary Reference Intakes for Japanese (2010) have given one age-class for elderly: 70 years and over (the below class is 50-69 years of age).

According to the recent study in the Netherlands, energy requirement seems to be stable up to around 53 years of age and to decrease hereafter depending on age. This is effected both by decrease in basal metabolic rate and in physical activity. This result suggests the need to make age-class narrower than the present one. However, this indicates, at the same time, the difficulty of research for this purpose because it also indicates the necessity of a large number of subjects to measure. In addition to this difficulty, a work burden is heavier for field workers and interviewers in the studies for the elderly than those for young and middle-aged subjects.

* M.D., Ph.D. Department of Social and Preventive Epidemiology, the University of Tokyo

Self-recording of physical activity may also be difficult for elderly subjects. There are thus several problems to resolve.

Can we establish estimated energy requirement in the Dietary Reference Intakes for Japanese elderly? For this, research techniques of epidemiologic studies on energy intake and expenditure, and physical activity should urgently be developed, and large-scale studies with high quality should accordingly be performed

Activity Energy Expenditure and Body Composition throughout the Adult Life Span

Klaas R Westerterp*

Abstract

Associations between body composition and the energy expended on maintenance and activity are complex and age dependent. Here, associations are examined between body composition and daily (DEE), basal (BEE) and activity energy expenditure (AEE) throughout the adult lifespan. Firstly, data are presented on a cross sectional analysis of 529 adults (18 to 96 y). Subjects had DEE measured using doubly-labeled water and body composition using isotope dilution. AEE was calculated as 0.9DEE - BEE and Physical activity level (PAL) from DEE/BEE. Up to age 52 fat-free mass (FFM) and fat mass (FM) were positively associated with age in males but there was no significant effect in females. There were no effects of age on DEE and AEE. Average DEE in males (14.1 MJ/day) was 27% greater than in females (10.7 MJ/day). PAL averaged 1.84 in males and 1.75 in females. Above the age of 52, FFM, FM, DEE, BEE and AEE were all negatively associated with greater age. The effect of age on AEE was greater than on BEE, consequently PAL by the age of 95 was only 1.36. PAL and AEE were both unrelated to FFM (both age adjusted). In conclusion, although older individuals aged < 52 y tended to be fatter, physical activity level and AEE were not associated with age. The lower AEE and BEE in older subjects aged > 52 y meant PAL was lower in older individuals. An absence of a relation between age-adjusted PAL and FFM suggested that greater physical activity was not associated with higher FFM in the elderly. Secondly, the effect of exercise training was evaluated from studies in which DEE was measured with doubly-labeled water before and at the end of the training program. Before the training, PAL showed and age dependency as described above. Initial PAL values were around 1.7, with lower values for subjects over age 60. It was intriguing to observe that the PAL level of younger subjects was modified with exercise training to values around 2.0, while exercise training had no effect in older people. Older people compensated for the training exercise with a reduction of physical activity in the non-training time. Thus, it seems the flexibility of PAL in older subjects is limited.

Introduction

Daily energy expenditure (DEE) has three components: basal energy expenditure (BEE); diet induced thermogenesis (DIT) and activity induced energy expenditure (AEE). Basal energy expenditure is determined by fat-free mass (FFM) and thus a function of body size. Diet induced thermogenesis is determined by food intake and diet composition. It comprises about 10% of energy intake for the average diet with 10-15% energy from protein, 30-35% energy from fat and the remaining part from

* Professor, Dr., Health, Medicine and Life Sciences, Human Biology, Maastricht University

carbohydrate [1]. Thus DIT is 10% of energy expenditure when one eats according to what one needs. Activity energy expenditure is a function of body size and body movement. It is the most variable component of DEE and can be calculated as 0.9 times DEE, assuming 10% of DEE is DIT, minus BEE: AEE = 0.9DEE - BEE. Alternatively, the physical activity level (PAL) of a subject can be calculated by adjusting DEE for differences in BEE as: PAL = DEE/BEE. Daily energy expenditure decreases with aging through a reduction of BEE and a reduction of physical activity. Questions are whether the age induced changes in DEE can be delayed or prevented. Firstly, how and why does DEE change with increasing age? Secondly, is the change in DEE delayed in subjects with a high habitual activity level? Finally, is it possible to prevent the age-induced changes as described with exercise training?

Energy expenditure under daily living conditions is nowadays measured with doubly labeled water. A typical observation interval is one week for highly active subjects up to three weeks for very sedentary subjects[2]. Here, data are presented as published since the first application of the technique in man in 1982[3]. Energy expenditure, more specifically BEE is related to body composition as measured with Deuterium dilution.[4]

Energy expenditure and age

The first compilation of human energy expenditure data as assessed with doubly-labeled water was an analysis of 574 measurements in 1996.[5] The review describes the levels of free-living energy expenditure in people from affluent societies as affected by body weight, height, age and gender. Conclusions were that absolute levels of energy expenditure whether expressed as DEE, BEE or AEE rise with increasing body size, peak in young adult years and decline thereafter. Adjusted for body size, energy expenditure declines with age throughout life. We recently published a cross-sectional study in 529 adults aged between 18 and 96 y[6]. There was a strong difference in the effect of age on body composition and energy expenditure at different times of life. It appeared there was a transition around the age of 40 to 60. We identified the exact position of these transitional breakpoints in the data using segmented regression analysis on the data separated by sex. In all cases the incorporation of a breakpoint into the analysis significantly improved the fit of the data above a simple linear regression with age as the predictor. To facilitate comparisons between the sexes we took the average of these breakpoints (52 y) and analyses were performed separately for a younger cohort of subjects aged 18-52 y, consisting 166 women and 185 men, and an older cohort of subjects aged 52.1 y and over, involving 74 women and 104 men.

Up to the age of 52 there were large gender related differences in body composition and energy expenditure. On average females under the age of 52 had FFM of 47.2 kg (sd = 7.0) while in males FFM was 30% greater and averaged 62.5 kg (sd = 8.4) ($p < .001$). Both FFM and fat mass (FM) were significantly positively associated with subject age between 18 and 52 y in females and in males. As both FFM and FM were higher in older subjects, BMI was also positively related to subject age. At age 45 almost half the subjects were obese (BMI > 30). It is well established that greater FM is associated with elevated FFM. Daily energy expenditure in males under 52 averaged 14.1 MJ/day (sd = 2.6) while in females the average was significantly lower by 27% at 10.7 MJ/day (sd = 1.9). This difference of 3.4 MJ/day in daily energy expenditure was contributed to by significantly greater basal and activity energy expenditure in the males. Hence in the females basal energy expenditure averaged 6.2 MJ/day (sd = 1.0) while in the males it averaged 7.6 MJ/day (sd = 1.2). The difference in activity energy expenditure between males and females was even greater. Hence females expended on average

3.4 MJ/day (sd = 1.1) on activity energy expenditure compared with 5.0 MJ/day (sd = 1.8) in males. This difference contributed 60% of the overall difference in daily energy expenditure between the sexes. At the individual level basal energy expenditure was significantly positively related to FFM. There was no significant relationship between the FFM and PAL in subjects aged 18 to 52 y in males and in females there was a statistically significant but very weak positive relationship. Individuals aged 18-52 y with greater FFM (independent of FM) were generally not more physically active.

Above the age of 52 y in both males and females greater subject age was associated with lower FFM and FM. Moreover the lower FFM in older subjects remained when it was adjusted for differences in FM. On average FFM in the females aged > 52 y was lower by 0.13 kg/y (95% CI: 0.05 to-0.20 kg/y) while in men it was lower by on average 0.42 kg/y (95% CI: -0.32 to -0.53 kg/y). In the very oldest individuals aged 90-100 y the average sex difference in FFM was less than 10 kg, compared with a 15 kg difference in subjects aged 18-52. The lower basal energy expenditure in older subjects in this age group appeared solely due to older subjects having lower fat and fat-free body masses.

Body composition and habitual activity level

Activity energy expenditure was significantly lower in older subjects. On average the PAL between ages 18 and 52 y averaged 1.84 in males and 1.75 in females. In the subjects aged 90-100 this averaged only 1.36 in both sexes. Above the age of 52 both FFM and PAL were generally lower in older individuals, although early in this period an age effect was less noticeable, particularly in males. There was no significant relationship between age adjusted FFM (also adjusted for FM) and age adjusted PAL. Consequently individuals in this older age cohort with greater FFM for their age and body fatness were not more physically active. Alternatively greater physical activity at any particular age was not associated with higher FFM.

The lower levels of physical activity in older subjects who also have reduced fat-free mass has led to suggestions that these phenomena are causally linked, either because elevated physical activity protects against age related FFM loss, or because preserved FFM loss allows greater physical activity. The data presented here do not support this interpretation because once the confounding influence of age had been removed we found no association between FFM and PAL. This analysis is consistent with previous studies based on a smaller sample size and studies measuring physical activity by accelerometry or by questionnaire rather than directly. Consistent with our correlational findings other studies have shown that physical activity interventions may alter muscle function and increase fat loss, but do not halt the progress of muscle mass loss in the elderly.[7] Generally, it is now regarded that lowered physical activity owes more to changes in muscle composition and physiology than quantity and that sarcopenia is caused by a complex interaction of nutritional factors such a protein intake, oxidative stress, inflammatory changes and hormonal effects.[8,9] For a person at any given age (> 52), however, there is no association between how much energy they expend on physical activity and how much fat-free mass they have, suggesting physical activity does not protect against loss of fat-free mass, and that loss of fat-free mass is not associated with lower physical activity levels.

Energy expenditure and exercise training

Physical activity declines with age. Black et al. concluded from an analysis of 574 doubly-labeled water measurements that the physical activity level for females is fairly constant between 13 and 64 years, and lower at younger and older ages[5]. For males physical activity rises to a peak at 18-29 years and declines thereafter. Starling et al.

reported a physical activity level of 1.68±0.28 in a group of nearly one-hundred 69±8 y subjects with no significant difference between women and men.[10] Westerterp and Meijer reported a physical activity level of 1.76±0.20 in 20-34 y subjects, 1.79±0.25 for a 35-49 y group (no difference), 1.62±0.26 for a 60-74 y group (lower, $p<0.001$), and 1.31±0.24 for 75 y and older (lower, $p<0.0001$).[11] There seems to be a gradual decline with age, starting at about age 50 and getting more pronounced after age 80. A physical activity level of 1.67 denotes an activity associated energy expenditure of 30% of total energy expenditure. Thus, on average, subjects of 65 y and over spent less than 30% of daily energy expenditure on physical activity. Subjects of over 80, generally have an extremely low level of physical activity, well below the level of 1.5 as defined for sedentary adults. It is intriguing to observe that the physical activity level of younger subjects was modified with exercise training while exercise training had no effect in older subjects.[12] In the elderly, exercise training does not result in an increase activity level through a compensatory decrease of non-training activity.[13] However, training has positive effects on fitness, endurance, range of motion, and balance control.[14] Additionally, aerobic training has positive effects on brain function including functional connectivity.[15]

In conclusion, Greater physical activity is not associated with higher fat-free mass in the elderly. In the elderly, training activity is compensated by a decrease in non-training activity. Exercise training does result in improved physical fitness and improved brain function.

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Effects of exercise training-induced hormonal changes on body composition

Tetsuya Izawa*, Jun-etsu Ogasawara**, Takuya Sakurai**,
Takako Kizaki**, Hideki Ohno***

1. Introduction

Chronic exercise training (TR) is well known to change body composition with either the reduction of body fat mass or the increase in lean body mass (LBM), or both. The later phenomenon mainly results from an increase in muscle mass, and the former, the reduction of body fat mass is accompanied by some biological adaptations of adipocytes to TR[1)]. TR-induced reduction in fat mass is evidently associated with the reduction of adipocyte size, and the potential increase in adipocyte lipolytic activity provides one of mechanisms for exercise training-mediated decrease in adiposity. Multiple previous studies have indicated that adipocyte size per se may be one of the critical regulators of several biological functions of adipocyte, e.g. adipocyte lipolysis, leptin and/or adiponectin mRNA expression and/or protein secretion. On the other hand, it is well known that TR in the early life of rats inhibits the normal increase in body weight and that this phenomenon results from the smaller adipocyte size, the lower content of triglyceride per adipocyte and/or the fewer number of adipocytes in TR rats than in freely eating sedentary control (CR) rats[2)]. Under the condition where TR induced the fewer number of adipocytes, TR suppressed the adipogenic responses of stromal vascular fraction (SVF) cells containing adipose tissue-derived stem cells and preadipocytes, and the underlying mechanisms involve up regulation of hypoxia inducible factor-1α (HIF-1α) [3)].

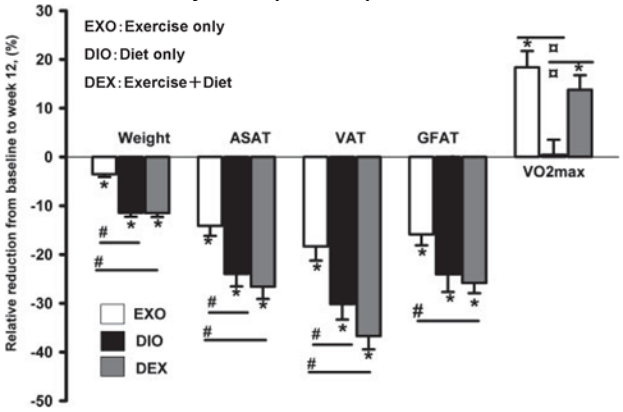
2. Visceral adipose tissue (VAT) and subcutaneous adipose tissue (SAT)

White adipose tissue (WAT) is dispersed throughout the body with major intraabdominal depots around the omentum, intestines, and perirenal areas, as well as in subcutaneous depots in the buttocks, thighs, and abdomen, and the biological functions and the expressions of genes are different between VAT and SAT. For example, the lipolytic response of adipocytes to catecholamines is higher in VAT than in SAT, but the secretion of leptin higher in SAT than in VAT. Moreover, the magnitude of the developmental genes expressions is different between VAT and SAT in humans and animals, and in human adipose, there were very strong correlations of Gpc4 expression with BMI and WHR in both males and females[4)]. In this case, the correlation in the two depots was in opposite directions, with decreasing Gpc4 expression in SAT with increasing BMI and WHR and increasing Gpc4 expression in VAT with increasing BMI and WHR. been examined and the enormous evidence Thus,

*Department of Sports Biochemistry, Graduate School of Health and Sports Science, Doshisha University
**Department of Molecular Predictive Medicine and Sport Science, Kyorin University, School of Medicine

genetically programmed developmental differences in adipocytes and their precursors in different regions of the body have been suggested to play an important role in obesity, body fat distribution, and potential functional differences between VAT and SAT.

Figure 1 Changes in body weight, fat distribution, and VO_2 max after the 12-week intervention in obese subjects (Ref. 5).



Unfortunately, it is also apparent that in sedentary middle-aged men and women, short periods of physical inactivity lead to significant weight gain, substantial increases in VAT, and further metabolic deterioration. On the other hand, there is a dose–response relation between amount of exercise and changes in VAT, and the relative reduction in VAT in response to TR is greater than that in SAT (Fig. 1)[5,6]. Weight loss is similar when the same degree of negative energy balance is produced by diet alone compared with exercise alone, but diet alone is unable to enhance cardiovascular system. In a recent review[7], evidence now supports the conclusion that there is a dose–response relationship between exercise amount and these changes, i.e., more exercise leads to additional benefits. Additionally, there are a number of important cardiometabolic risk factors that were most favorably affected by moderate-intensity compared to vigorous-intensity exercise. However, it remains unknown how TR dose-dependently mediate the reduction of VAT and SAT and why the reduction of VAT is more sensitive to TR than that of SAT being. Although it remains unknown whether the developmental genes play a role in these phenomena, one of explanation for the later phenomenon may be that the ability of VAT to mediate energy homeostasis is greater than that of SAT[8]. In this context, the phenomenon indicates that TR-induced reduction is due to the reduction of adipocyte size that results from the reduction of lipid droplet.

3. The relationship between adipocyte size and its biological function

In sedentary individuals of similar age, both basal and hormone-stimulated adipocyte metabolism increase with adipocyte size[2]. Moreover, a positive correlation between adipocyte size and its ability to secrete many pro- and anti- inflammatory cytokines, including leptin, interleukin-6, tumor necrosis factor-α, and adiponectin[9,10]. In rat epididymal and inguinal adipose tissue, positive relationships between adipocyte size and both leptin and adiponectin mRNA expression were found (Fig. 2)[9]. Comparison of TR and CR rats showed no significant effect of TR on the slopes of the linear regression lines of correlation between leptin mRNA and adipocyte size in either adipose tissue, whereas the slopes of the regression line of correlation between adipocyte size and adiponectin mRNA were greater in TR group. Thus, TR-induced reduction in leptin mRNA expression was closely associated with smaller adipocyte size. However, TR amplified the adipocyte-size-dependent expression of adiponectin mRNA, suggesting that TR-induced alterations in adiponectin mRNA may also be mediated by factor(s) other than adipocyte size. On the other hand, as described above, both basal and hormone-stimulated adipocyte metabolism increase with adipocyte size[2]; nevertheless, the smaller size of adipocyte in TR subject exhibits the enhanced ability of adipocyte lipolysis. The mechanism by which TR enhances adipocyte lipolysis has extensively has been accumulated.

Figure 2 Correlation between adipocyte size and leptin and adiponectin mRNA expression, respectively (Ref. 9).
C: Control rats; TR: trained rats *P<0.05 or less

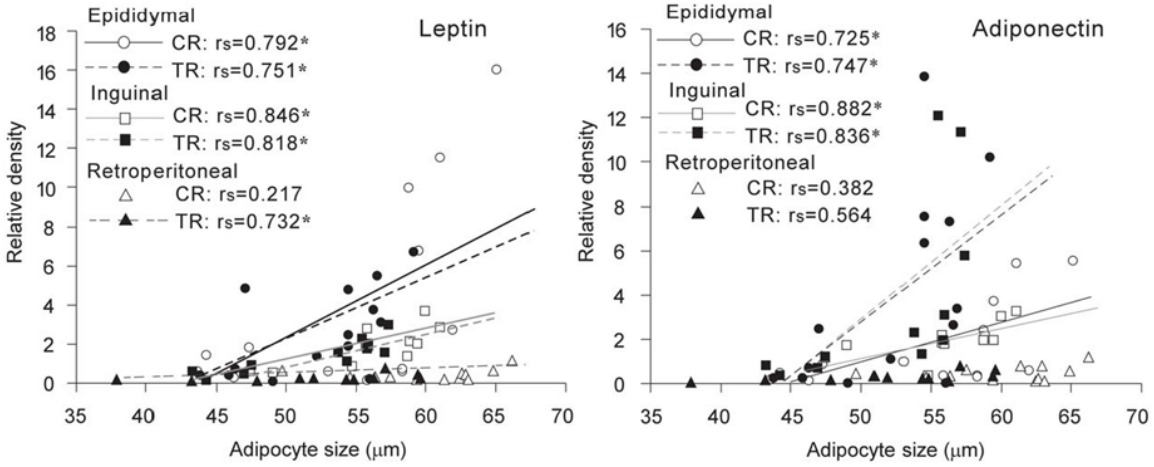
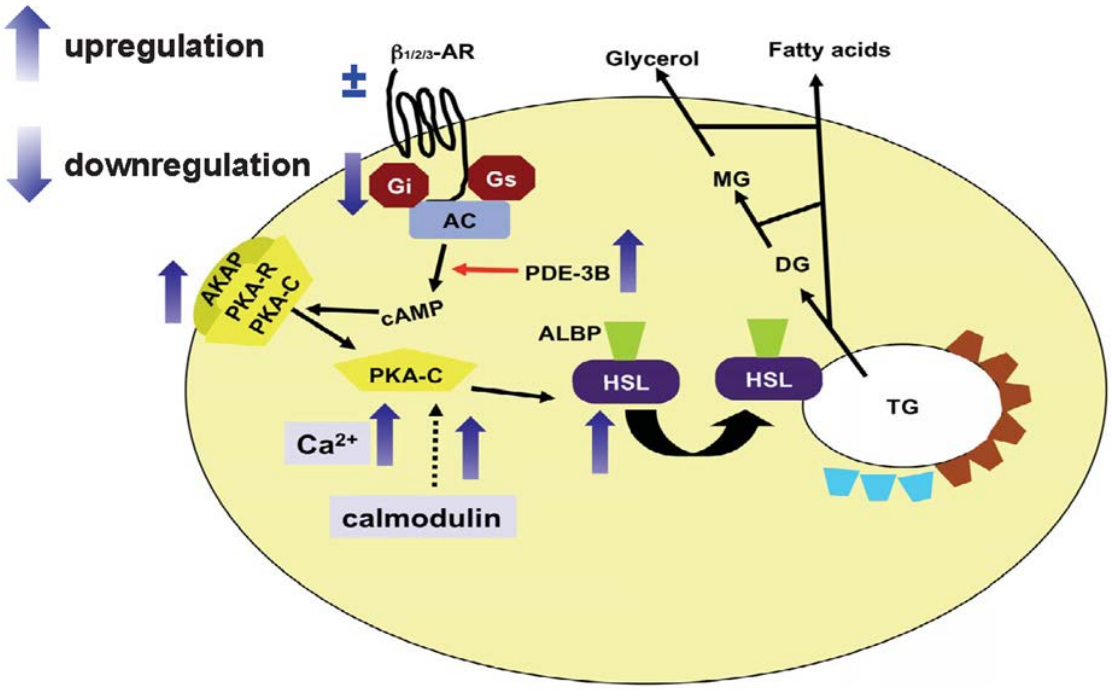


Figure 3 Exercise training-enhanced adipocyte lipolysis results from changes at the some sites of lipolytic cascade.



4. Lipolytic cascade

It is widely accepted that the hormonal activation of adipocyte lipolysis is mediated via a cAMP-dependent process. Increased concentrations of cAMP activate cAMP-dependent protein kinase (PKA), which, in turn, leads to phosphorylation and activation of hormone-sensitive lipase (HSL). This lipolytic cascade is stimulated by several hormones, such as catecholamines, ACTH, growth hormone and thyroid hormone. Moreover, human adipocyte is able to respond atrial natriuretic peptide (ANP). Catecholamines, adrenaline and noradrenalin stimulate adipocyte lipolysis via a β-adrenergic receptor (β-AR). β-AR is coupled with the stimulatory G-protein (Gs), which, in turn, activates adenylate cyclase (Fig. 3). The activation of adenylate cyclase leads to the increases in cAMP production. Increased concentrations of cAMP activate cAMP-dependent protein kinase A (PKA).

The regulatory unit of A-kinase is occupied with cAMP, and, in turn, the catalytic subunit is activated. The activated catalytic subunit leads to phosphorylation and activation of HSL. Finally, HSL translocates to HSL and degrades triacylglycerol inside lipid droplets. Alpha2-AR is coupled with the inhibitory G-protein (Gi), resulting in the inhibition of cAMP productions. Adenosine and prostaglandin receptors are also coupled with Gi. These substances increase progressively during lipolysis, and then inhibit lipolysis in an autocrine fashion. ANP stimulates protein kinase G (PKG) through cGMP productions, and in turn PKG activates HSL.

Accumulated evidence shows that a redistribution of HSL from cytosol to lipid droplets and adipose triacylglycerol lipase (ATGL) are required for the subsequent activation of full lipolysis[11]. Under basal conditions, non-activated perilipin (PLIN) coats the surface of a single triacylglycerol (TAG) lipid droplet and binds to the comparative gene identification-58 (CGI-58), and HSL is also dispersed in the cytoplasm. When cAMP activates PKA as described above, PKA leads to phosphorylation of both PLIN and HSL, and in turn the phosphorylated PLIN induces physical alterations of the lipid droplet surface, which facilitate the action of phosphorylated HSL on TAG hydrolysis. Phosphorylation of PLIN also promotes CGI-58 release, which, in its "free" form, binds to ATGL and activates TAG hydrolytic function of the enzyme, thus resulting in diacylglycerol (DAG) production. HSL and monoacylglycerol (MAG) lipase contribute to the final hydrolysis of DAG and MAG, respectively. Concomitant enhancement of ATGL, HSL and MGL activities is absolutely necessary for the complete hydrolysis of TAG and the release of non-esterified fatty acids and glycerol.

5. The mechanism by which TR enhances adipocyte lipolysis

Numerous in vitro studies show that TR enhances the lipolytic response of adipocytes to catecholamines in laboratory animals and humans. TR induces the enhanced efficiency of the coupling between β-AR and Gs, the reduction of the Gi protein and the enhanced ability of HSL to translocate lipid droplets (Fig. 3) [1,12,13]. Moreover, a recent study shows that when glycerol release was significantly elevated immediately (0h) and three hours (3h) after exercise, both activity of HSL and its localization to the pellet were significantly greater in the pellet fraction, which is included in lipid droplet associated-proteins, than in the supernatant fraction[14]. In the pellet fraction, although neither perilipin A nor CGI-58 protein level changed, level of perilipin A/CGI-58 complex was significantly reduced, accompanied by up-regulated association of perilipin A/HSL at 0h and 3h after exercise. In addition, a possibility is shown that TR can enhance the level of perilipin A/CGI-58 complex (Ogasawara et al., submitted)

6. ANP mediates lipolysis

As described above, ANP has a strong lipolytic effect, and it is shown that in overweight men, the lipid-mobilizing effect of ANP was markedly enhanced after TR as was that of isoproterenol (Fig. 4)[15]. Exercise induced an approximately twofold increase in ANP levels, and plasma ANP concentration increases in response to the intensity. On the other hand, plasma adrenalin level increases extensively over about 60% of VO2max. Moreover, in comparison with isoproterenol, ANP induces a strong lipolytic effect. Thus, lipolysis during low-to-moderate exercise may depend greatly on ANP-mediated one.

7. Aging suppresses adipocyte lipolysis

It has been shown that the rate of catecholamine-stimulated lipolysis was reduced in the elderly subjects, and that maximal lipolytic responses of SAT cells to isoproterenol (β-agonist) and to postreceptor agents such as dibutyryl cAMP, forskolin, and theophylline were lower in middle-aged than in young men (Fig. 5)[16].

Figure 4 Exercise training enhances ANP-induced lipolysis in obesity human (Ref. 14).

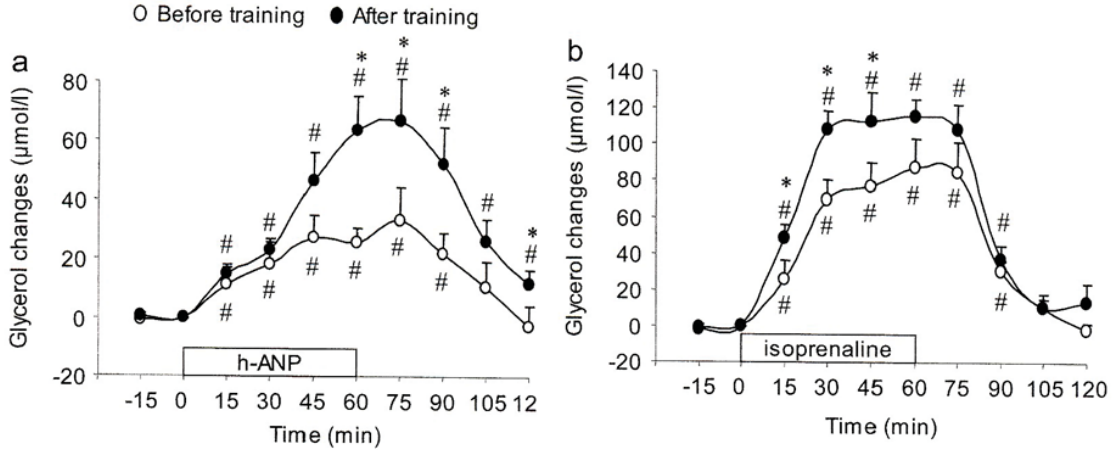
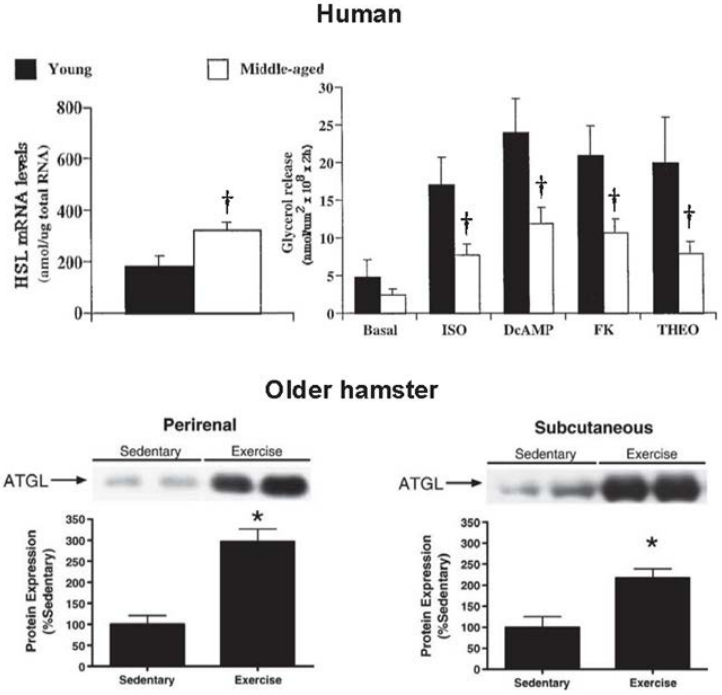


Figure 5 Aging enhances the mRNA expression of hormone sensitive lipase (HSL) (the upper left panel) but blunts maximal lipolytic responses of subcutaneous abdominal adipocytes at the post receptor sites (the upper right panel) (Ref. 15). Moreover, the protein expressions of adipocyte triglyceride lipase (ATGL) increase in older hamster (the lower panels) (Ref. 16). † P<0.05 or less vs. young, ∗ P<0.05 vs. sedentary.



Because mRNA levels of HSL[16] and the protein expressions of ATGL[17] were higher in middle-aged and older hamster, respectively, than in young men and hamster, a possible interaction of HSL with PLIN and/or that of HSL/ATGL with lipid droplets could be altered with advancing age. Aging, together with decrease in lipolytic hormone, leads to a prevalence of liposynthetic activity at the visceral level both in men and in women (Fig. 6) [18]. In women the phenomenon has an abrupt onset after the menopause. Under the condition, the ability of growth hormone to suppress lipogenesis is also depressed. Thus, fat accumulation proceeds with aging.

From this point of view, the degradation and burning of fat by exercise may be required in older subjects. As shown in Fig. 7, during exercise, adrenalin concentration was

higher in middle-aged trained cyclists than in middle-aged sedentary men from 15 minutes to the end of exercise above ventilation threshold[19]. In addition, the release of ANP after a 10-km run was greater in older (41-55 yr of age) than in younger (24-34 yr of age) individuals[20]. Thus, the secretary responses of some hormones to exercise may be higher in older than in younger subjects, although there are the studies showing the contrary results. Administration of GH in supraphysiological doses to both obese women and to healthy elderly men decreases body fat and increases LBM. The combined lipolytic effect of GH and exercise, evidenced by increased plasma glycerol and serum NEFA concentrations, has been shown to be 3-fold greater than the effect of exercise alone (Fig. 8), although this increased substrate availability did not result in increased whole body fat oxidation (indirect calorimetry)[21]. Unfortunately, it should be noted that the changed lipid metabolism during exercise observed after endurance training alone or after endurance training combined with GH administration is not due to alterations in subcutaneous abdominal adipose tissue metabolism in elderly women[22].

Figure 6 Balance between visceral fat accumulation and mobilization hormones in men and women relative to aging in men and to both ageing and menopause in women. Ageing, together with decrease in lipolytic hormone, leads to a prevalence of liposynthetic activity at the visceral level both in men and in women. In women the phenomenon has an abrupt onset after the menopause (Ref. 17).

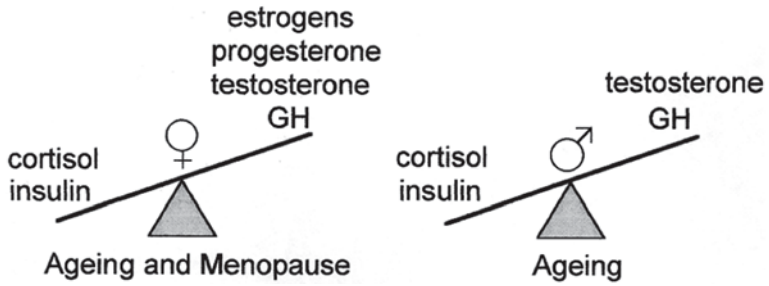
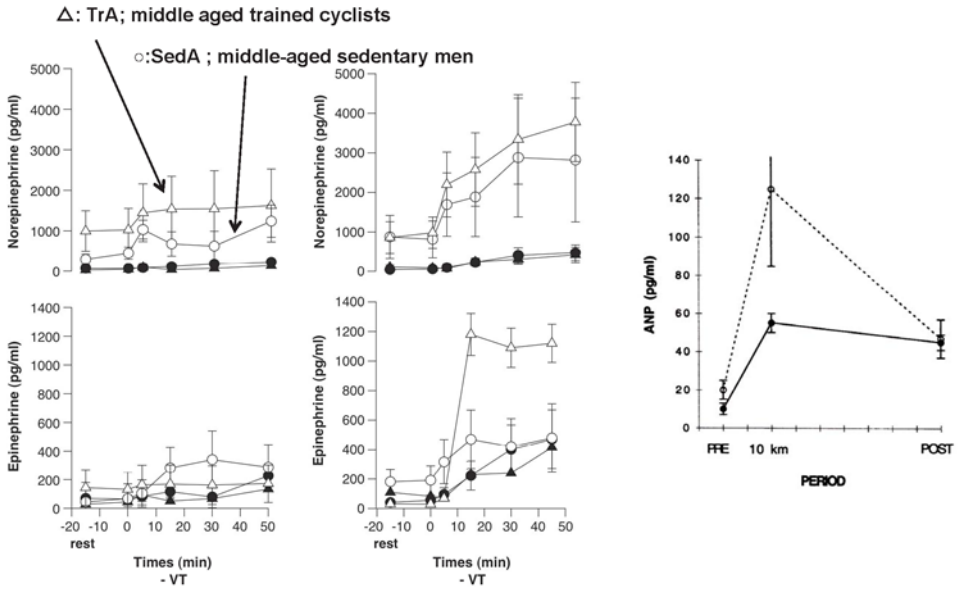
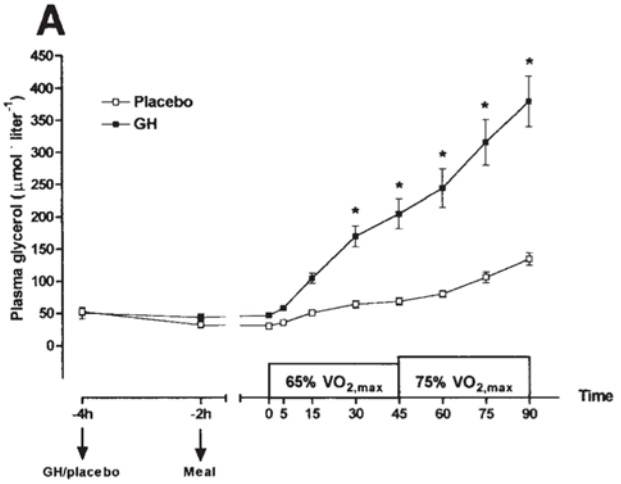


Figure 7 Plasma catecholamine concentrations in response to exercise are higher in older men than in young men (the left and middle panels) (Ref. 16). The release of ANP after a 10-km run is greater in older (41-55 yr of age) than in younger (24-34 yr of age) individuals (the right panel) (Ref. 19). VT: Ventilation Threshold.



Thus, there is a report showing no significant effect of GH administration on lipolysis in elder women, but GH response to acute exercise may increase lipolysis as an energy resource during recovery from exercise[23]. It can not be denied that aerobic TR combined with resistance TR, which is able to increase GH levels, might be useful strategy to alter body composition in elderly subjects.

Figure 8 Growth hormone augments exercise-induced lipolysis in trained young men (Ref. 20).



8. TR may suppress inflammation-related adipokine levels in WAT

Excess nutrients, in particular fat and lack of exercise play in the overall physiological process leading to obesity with hypertrophy of adipocytes. Recently, it has been suggested that proceeding of adipocyte hypertrophy leads to low-grade chronic inflammatory response of WAT[24]. Indeed, the expressions of the pro-inflammatory adipokines, such as tumor necrosis factor-α (TNF-α) and monocyte chemoattractant protein-1 (MCP-1), are upregulated in the adipose tissue of obese subjects[25]. Then, exposure of adipocytes to TNF-α and MCP-1 causes blunting of the insulin signaling. In this context, current literature reports that TR can decrease expression of inflammation-related adipokines: manganese-containing SOD (Mn-SOD) levels in adipocytes are significantly greater in TR rats compared with those in untrained rats, suggesting that TR reduces oxidative stress in WAT, thus leading to downregulation of inflammation-related adipokine levels in WAT. Moreover, TR suppresses the expression of inflammatory-related adipokines, including MCP-1 (Fig, 9) and F4/80 mRNA[26]. Similar finding has also been shown in humans, that TR inhibits inflammation in WAT via suppression of macrophage infiltration[27].

9. Effect of TR on adipogenesis

As discussed above, TR-induced reduction of fat mass is accompanied by reduced size of adipocytes accompanied by the enhanced lipolysis and downregulation of inflammation-related adipokine levels in WAT. Then, can TR also reduce the number of adipocyte via inhibition of adipogenesis? Human studies are difficult to reply this question. However, in animal studies, it has been shown that TR generally does not influence the number of adipocytes, but in rats, a reduction in the number of adipocytes may be induced by long-term training or by training commenced early in life[2]. Moreover, TR combined with diet-restricted reduced the number of adipocyte in rat inguinal WAT at 12 to 28 months of age[28].

We also confirmed that when TR was started from 4-5 weeks of age, a reduction in the number of adipocytes was found in epididymal WAT[3], and then tested the adipogenic responses of stromal-vascular fraction (SVF) cells containing adipose tissue-derived stem cells and preadipocytes in epididymal WAT from TR rats. In SVF cells of TR rats, expression of peroxisome proliferator-activated receptor γ (PPARγ) and PPARγ target lipogenic genes were dramatically downregulated, whereas that of preadipocyte factor-1 genes was significantly upregulated (Fig. 10). Lipid accumulation in SVF cells of TR rats after the induction of adipocyte differentiation was significantly suppressed in comparison to that of control rats (Fig. 11). Moreover, increased expression of hypoxia inducible

factor-1α (HIF-1α) protein was observed in SVF cells of TR rats. Pretreatment of YC-1, a potent HIF-1α inhibitor, in SVF cells of TR rats restored adipogenesis. Thus, TR suppresses the ability of SVF cells to differentiate into adipocytes, and that underlying mechanisms involve upregulation of HIF-1α expression.

HIF-1α increases vascular endothelial growth factor (VEGF)-A and VEGF-receptor expressions. In accordance with this well-known role of HIF-1α, the up-regulation of mRNA expression of Vegf-A and Vegf-receptor-2 were found in SVF cells of TR rats[29]. Moreover, TR significantly increased the number of endothelial cells per millimeter and per adipocyte (1.37- and 1.23-fold, respectively) in WAT. However, because the number of adipocytes was fewer while the number of endothelial cells was constant in the WAT of TR rats, the regression line of TR rats for adipocyte number-dependent endothelial cell number was shifted towards the left without significant differences in the slopes between groups.

Figure 9 Changes in MCP-1 protein in adipose tissue after exercise training (modified from Ref. 24). aP<0.05 vs. Control.

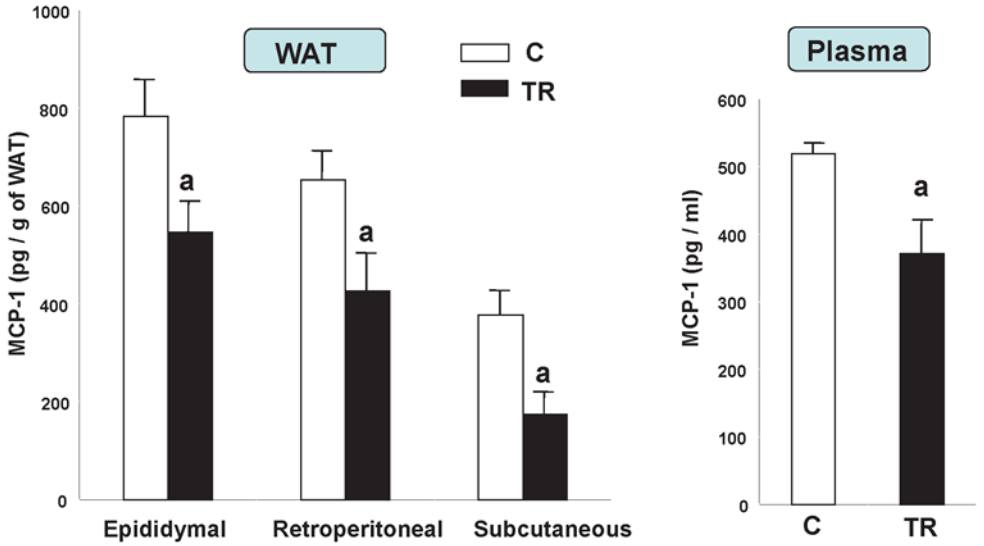


Figure 10 Exercise training alters the mRNA expressions of genes in stromal-vascular fraction cells (modified from Refs. 3 and 28). PPARγ : peroxisome proliferator-activated receptor γ, GPDH : glycerol-3-phosphate dehydrogenase, Pref-1: preadipocyte factor-1, HIF1-α, CEBP: CAAAT/enhancer binding protein, ACLP: aortic carboxypeptidase-like protein, VEGF: vascular endothelial growth factor, VEGFR: vascular endothelial growth factor receptor, C: control rats, TR: exercise training rats.

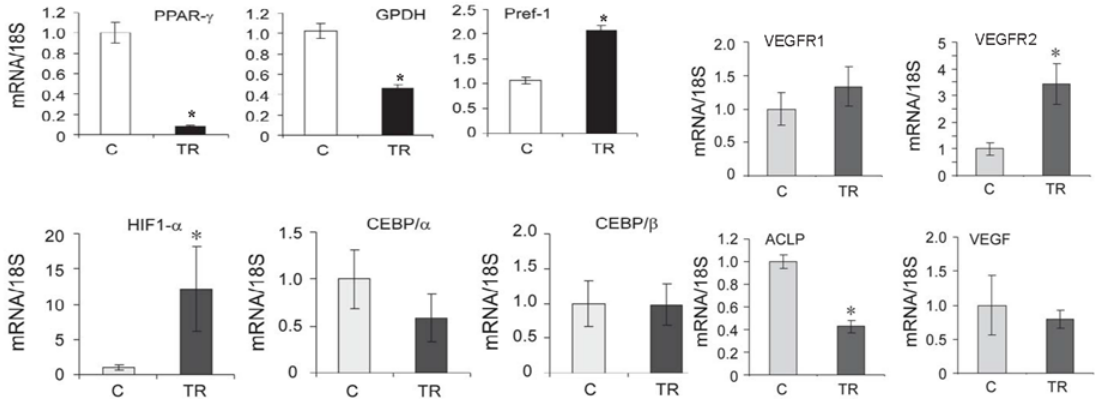


Figure 11 Exercise training inhibits adipocyte differentiation in SVF cells (panels A): Lipid droplets in SVF cells of C and TR rats after 7 days adipocyte differentiation were stained red using an Oil-Red-O solution. Whereas, YC-1, the HIF1-α inhibitor, enhances adipocyte differentiation determined by using an Oil-Red-O solution (panels B) with the increases in the mRNA expressions of adipogenic genes (panels C) (modified from Ref. 3). ACC: acetyl-CoA carboxylase, FAS: fatty acid synthase, LPL: lipoptrotein lipase , PPAR γ : peroxisome proliferator-activated receptor γ.

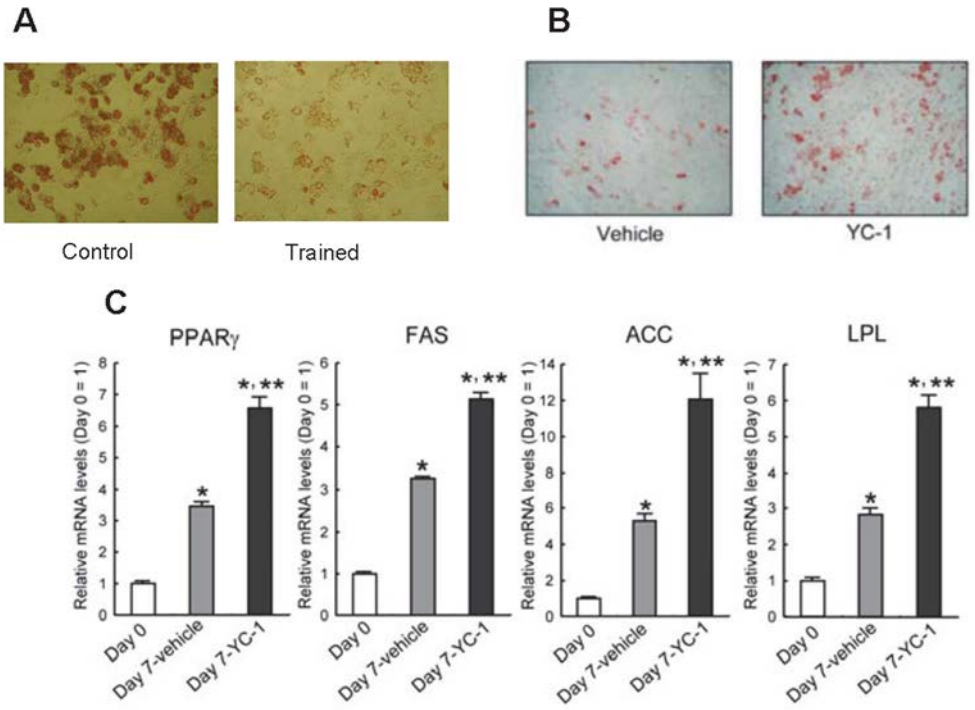
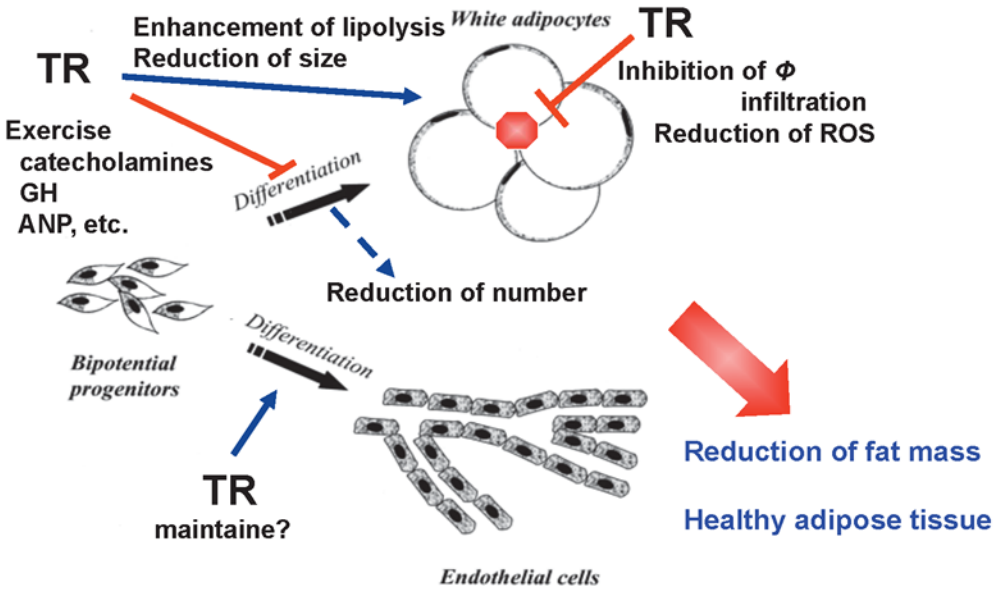


Figure 12 Biological and morphological adaptations of adipose tissue to exercise training.



10. Concluding remarks

We focused on the effect of TR on biological responses of adipocytes, which may be associated with TR-induced reduction in body fat. The outline is summarized in Fig. 12, although we did not discuss the interaction of TR-induced hormonal changes with changes in body composition in detail. There is growing evidence that obesity involves multiple genetic components that affect endocrine, metabolic, and regulatory mechanisms as well as environmental components doing so. Nehrenberg et al.[30] indicate that in mice, the effects of exercise on body composition depend on genetic background, and suggest that it is important to carefully consider genetic background and/or selection history when using mice to model effects of exercise on body composition. In human studies, a large portion of interindividual variation of exercise-induced fat oxidation goes largely unexplained (see Ref. [29], but this variation might also be explained by possible genetic differences among subjects. Further studies are required to consider individual differences for health-related TR responses that appear attributable to genetic variation.

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Hot Topics in Relation to Nutrients and ADL in Elderly People

Toshio Okano*

The more it becomes advanced age, the more it becomes easy to suffer a fall-induced fracture. Fracture in the elderly remarkably reduces activity of daily life (ADL), and often needs immense medical expense and prolonged life-support and life-care. Prevention of falls along with keeping bone mineral density normally is also one of the fundamental measures in the prevention of fracture. Two causes can be considered as a reason why a fall takes place to elderly people easily. One of them is a physical factor and it is a thing which cannot overcome by individual efforts and which happens in connection with aging, such as a decrease of locomotive function and loss of muscle strength, visual impairment, abnormalities in the sense of balance, a cardiopulmonary function fall, and a cognitive obstacle. Another is an environmental factor and it can be improved by individual efforts, such as housing environment, such as lighting, a level difference and an obstacle, and a floor on which it is easy to slide, drinking, medication, and malnutrition. A fall takes place by entangling two or more these factors in many cases. In recent years, the living guidance for elderly people, the improvement of housing equipment, etc. are progressing, but the measure from a nutrition side to elderly people is not necessarily performed fully.

In this review, I would like to discuss on the nutritional effects of vitamin D and vitamin K on bone health, focusing on locomotive diseases, especially bone fracture.

1. Main causes by which elderly people result in support required, requiring care, or death

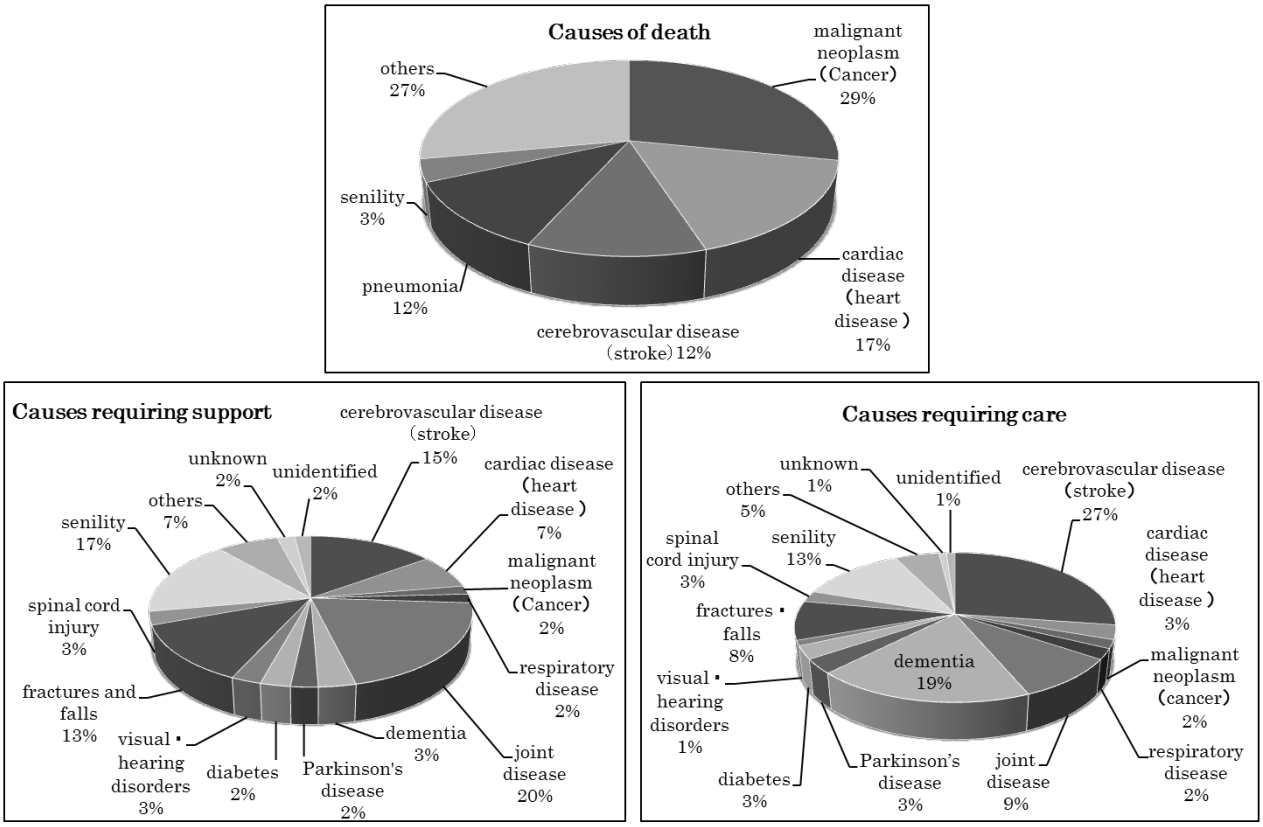
According to the Ministry of Health, Labour and Welfare vital statistics[1)], and National Livelihood Survey[2)] in the 2007 fiscal year, the 1st place to the 3rd place of a cause of death in our country were a neoplasm (cancer) (28%), cardiac disease (17%), and a cerebrovascular disease (12%), respectively. On the other hand, diseases for which support or care of a life is needed although it does not result in death were a cerebrovascular disease (27%), dementia (19%), a joint disease (9%), and fracture and a fall (8%) in requiring care, and a joint disease (20%), a cerebrovascular disease (15%), and fracture and a fall (13%) in requiring support. These figures show that the diseases which become a cause of death easily is not the diseases that not necessarily become support required and a cause requiring care, but the major cause for which brain and locomotive diseases bar independence of elderly people's life rather. Therefore, prevention of bone and joint diseases is important for keeping elderly people's ADL normally (Fig.1). There is a report that about 70% of fracture from which elderly people become "bedridden" was femoral neck fracture[3)], and not less

* Professor, Kobe Pharmaceutical University

than 90% of the cause was a fall[4].

In the femoral neck fracture patients, it is occasionally observed that their dietary intakes of energy and protein are about the halves of the requirements. In addition, there is a report that serum levels of albumin as a marker of malnutrition correlate with the situation of recovery after a femoral neck fracture operation, life prognosis and the onset frequency of complications[5]. Furthermore, there is a report that in the femoral neck fracture patients, their triceps skinfold thickness were thinner and their arm circumference length were shorter compared with those of the individuals of the same age. From such a background, the nutrition intervention trials aiming at elderly people's prevention from a fall has been conducted in every country in the world. However, according to the systematic review[6] using the number of fall persons or the number of falls as major outcomes, in most cases indicating that nutrition intervention was effective for the prevention of falls, patients were in severe malnutrition and given intensive medical care. This suggests that although malnutrition is a risk for falls and fractures, the risk of the falls and fractures which takes place to elderly people easily cannot be fundamentally reduced only by the improvement of nutrition. It is important for elderly people to take nutrients required for bone health positively.

Figure 1 Main causes of death, requiring support and requiring care in elderly people aged 65 and over



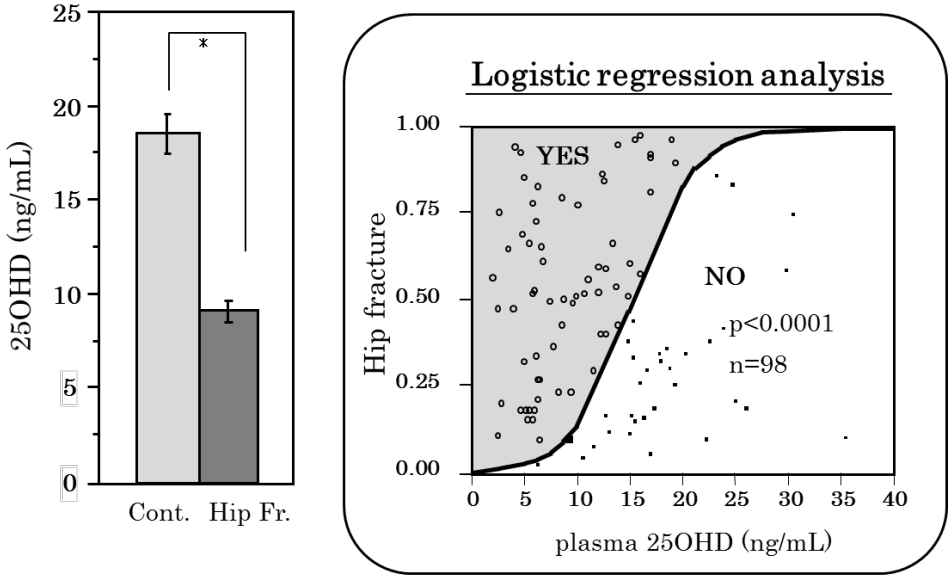
The figures based on the data from "Vital statistics 2007" and "Comprehensive Survey of Living Conditions of the People on Health and Welfare 2007" (The author made these figures based on the numerical values on the above literatures.)

2. Dietary factors affecting ADL in elderly people
- consideration from vitamins/minerals -

It is known that the pathological abnormalities of muscular tissues and loss of muscle strength happen with the abnormalities in bone calcification in rickets and osteomalacia. Since these symptoms are corrected by vitamin D medication, vitamin D is considered to play a role in maintaining the structure and function of skeletal muscle[7]. From this reason, supplementation of vitamin D is thought to be effective for prevention of falls. There are several reports indicating that body sway and frequency of falls are large and high in the individuals with vitamin D insufficiency[8-15] However, there is no report showing clearly that vitamin D has a fall preventive effect. We measured serum 25OHD concentrations of the female patients aged approximately 80 years who were sent to hospital for fracture, and compared with those of age-matched non-fracture women living in the same area[16]. Here, serum 25OHD concentration is a nutritional marker reflecting vitamin D status, and 20 ng/mL of serum 25OHD concentration was used as a cut-off value for vitamin D insufficiency/sufficiency. Serum 25OHD concentrations of fracture patients were approximately a half of the subjects not suffering a fracture, and logistic regression analysis revealed that there was strong negative correlation between serum 25OHD concentration and fracture in the patients with fractures (Fig.2). These results suggest that vitamin D deficiency is a risk for fracture.

To clarify why fracture becomes easy to happen when serum 25OHD concentration is low, we analyzed the relationship between serum 25OHD concentration and serum parathyroid hormone (PTH) concentration, lumbar bone mineral density, or a fracture prevalence rate in elderly subjects. As a result, it turned out that serum 25OHD concentration and serum PTH concentration show negative correlation, and bone mineral density falls as serum PTH concentration becomes high, and consequently fracture prevalence rate becomes high (Fig. 3).

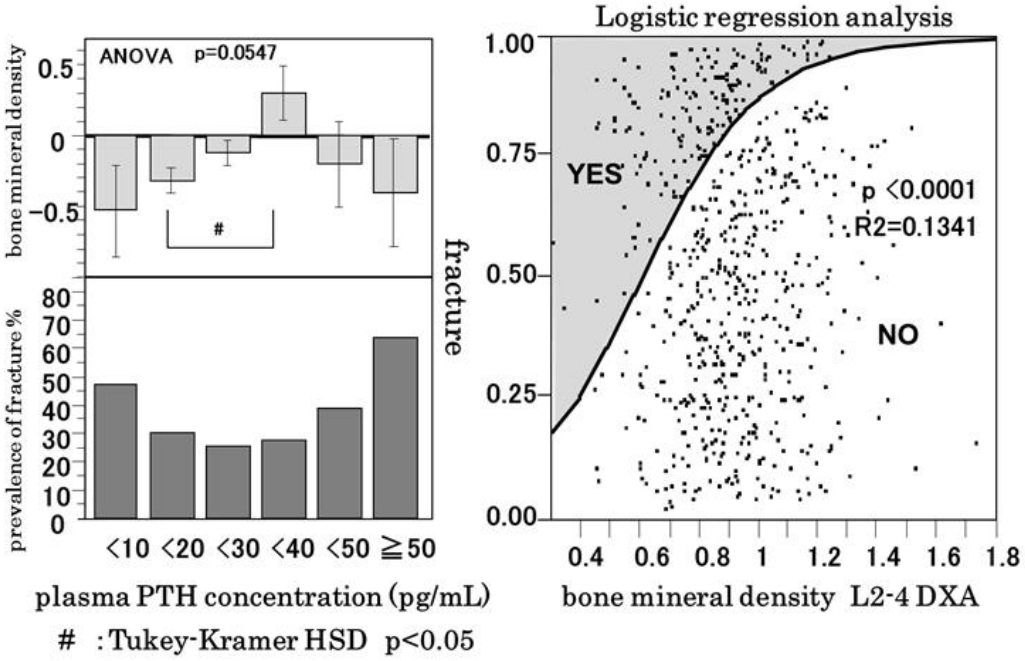
Figure 2 Comparison of plasma 25OHD concentrations between the subjects with non-fracture and femoral neck fracture, and association of plasma 25OHD concentration with femoral neck fracture



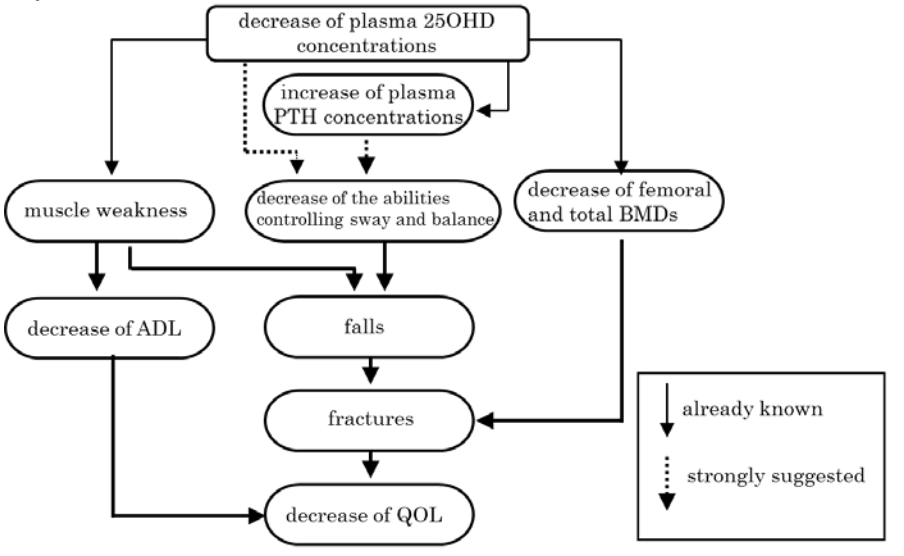
* Statistically significant p<0.0001 (Student's t-test)
Cont. : non-fracture
Hip Fr: femoral neck fracture

Suzuki et al has reported the relation between the increase of fall / fracture risk and the reduction of ADL/QOL due to the decreased serum 25OHD concentration as follows[17]. That is, if serum 25OHD concentration falls, then bone density will fall and it will make a bone vulnerable. In addition, it becomes easy for elderly to fall due to the decrease of muscular power, and the increase and decrease of sway and body capacity of balance conjointly. From this reason, vitamin D deficiency increase risk of falls and fractures in elderly people (Fig.4). In this figure, the scientific basis which

Figure 3 Relations between plasma PTH concentration and bone mineral density or prevalence of fracture



.Figure 4 The decrease of plasma 25OHD concentration enhances risk of falls and fractures, and consequently leads to the decrease of ADL/QOL in elderly people



Suzuki T. *Osteoporosis Jpn* 2003;11(4):797-804
(translated in English by the author.)

proves that the decrease of serum 25OHD concentration and the rise of serum PTH concentration increase body sway and reduces body capacity of balance is scarce.

There is a meta-analysis regarding the vitamin D intervention trials which make major outcome the fracture control effect for elderly people[18]. Vitamin D supplementation significantly reduced the fracture risk in both non-vertebral (Fig. 5) and femoral neck fractures (Fig. 6) in community dwelling subjects and nursing home residents.

Figure 5 Non-vertebral fracture reduction by oral vitamin D supplementation in community-dwelling and institutionalized older individuals. (Meta-analysis)

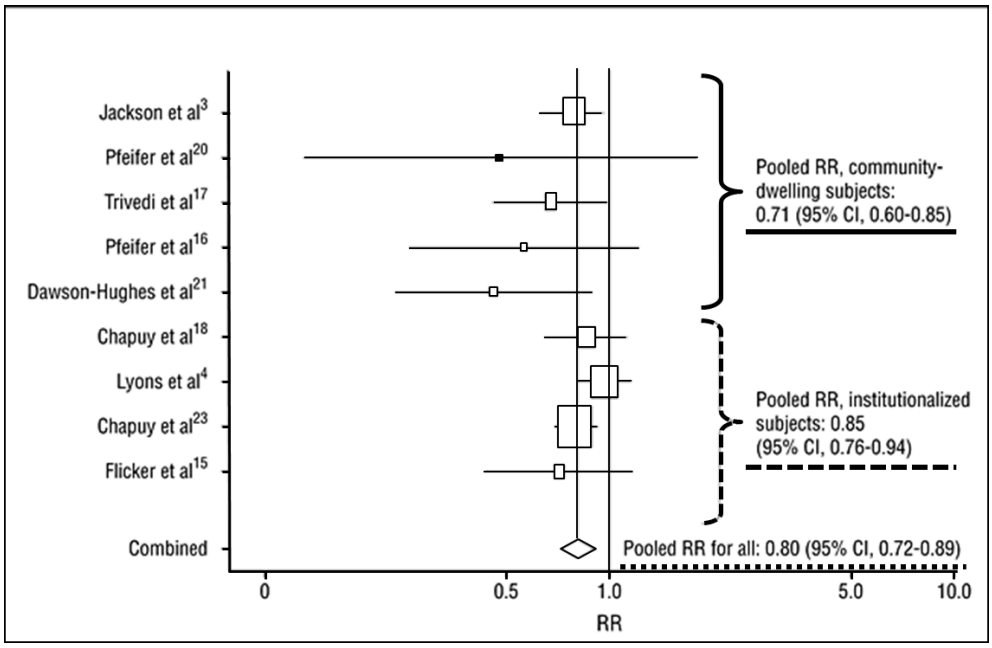
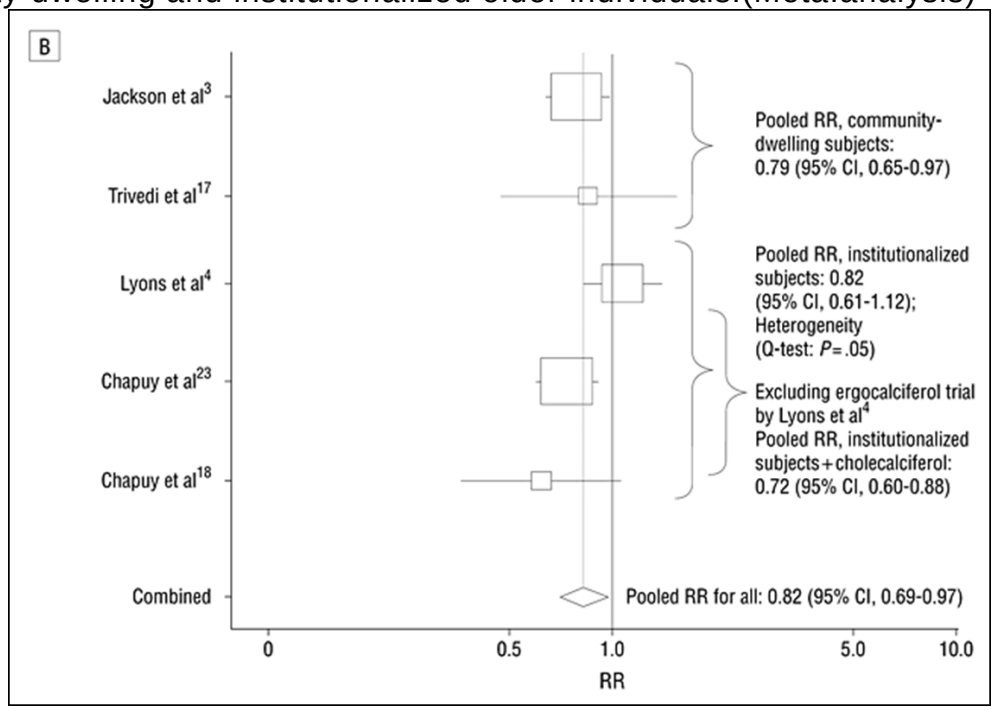


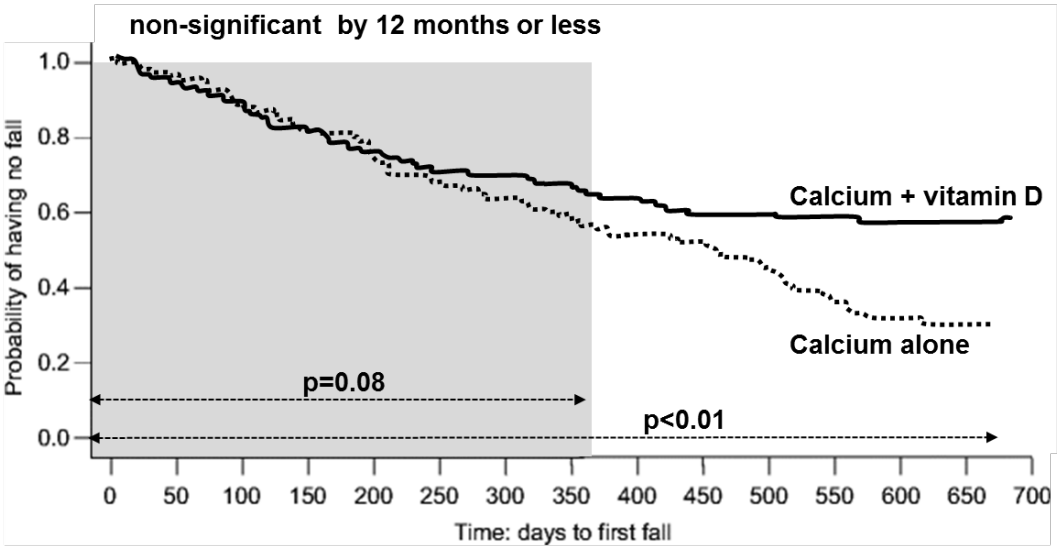
Figure 6 Hip fracture reduction by oral vitamin D supplementation in community-dwelling and institutionalized older individuals.(Meta.analysis)



Moreover, the combined use of calcium and vitamin D was more effective to reduce frequency of falls compared to calcium independent use in the long period of intervention (although the significant effect was not seen within one year, the significant effect was observed by the intervention in the period more than for one year) (Fig.7) [19]. Thus, although vitamin D supplementation may reduce fall / fracture risk in elderly people, most studies described here were conducted in Western countries other than Japan, and these results cannot be directly applied to Japanese people. Intervention trials for Japanese people are required.

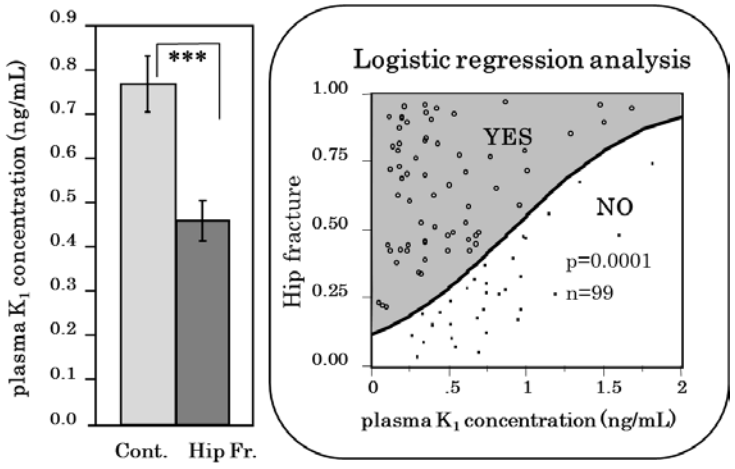
There are several epidemiological reports indicating that vitamin K is effective for prevention of fracture, and dietary intake of vitamin K and fracture rates correlates negatively[20-27]. We also examined the relation between serum vitamin K_1 concentrations and frequency of femoral neck fractures, and observed high correlation between the two (Fig.8)[16].

Figure 7 Falls in elderly subjects with calcium alone or calcium plus vitamin D for 12 months or more



Pfeifer, M., et al. *Osteoporosis Int,* 2009;20(2):315-322

Figure 8 Comparison of plasma K1 concentrations between the subjects with non-fracture and femoral neck fracture, and association of plasma K1 concentration with femoral neck fracture



* Statistically significant p<0.0001 (Student's t-test)
Cont. : non-fracture
Hip Fr: femoral neck fracture

In our country, vitamin K_2 is approved as a medicine for osteoporosis, and its fracture preventive effect is proved in post-menopausal women including osteoporotic patients (Fig.9)[28)]

Recently, the fact that various dietary vitamin K homologues are converted to menaquinone-4(MK-4) in the body, and stimulates the function of osteoblasts and promotes bone formation, has been reported (Fig.10)[29,30)]. It is proved that the enzyme which bears the conversion reaction of vitamin K homologues to MK-4 is ubiA prenyltransferase containing domain 1 (UBIAD1), and it has become clear that geranylgeranyl pyrophosphate derived from mevalonate pathway is used as the side chain of MK-4 (Fig. 11). MK-4 has the strongest biological activity among vitamin K homologues, and it has been clarified that MK-4 regulates transcription of genes as a specific ligand for nuclear receptor steroid and xenobiotic receptor (SXR) or related to the enzymes involved in the metabolism of lipids and sex hormones[31-34)]. The more the role of MK-4 in bone metabolism becomes clear, the more the role of vitamin K in bone health is expected to become clearer.

Figure 9 Prevention of bone fracture with vitamin K2 in post-menopausal women with osteoporosis

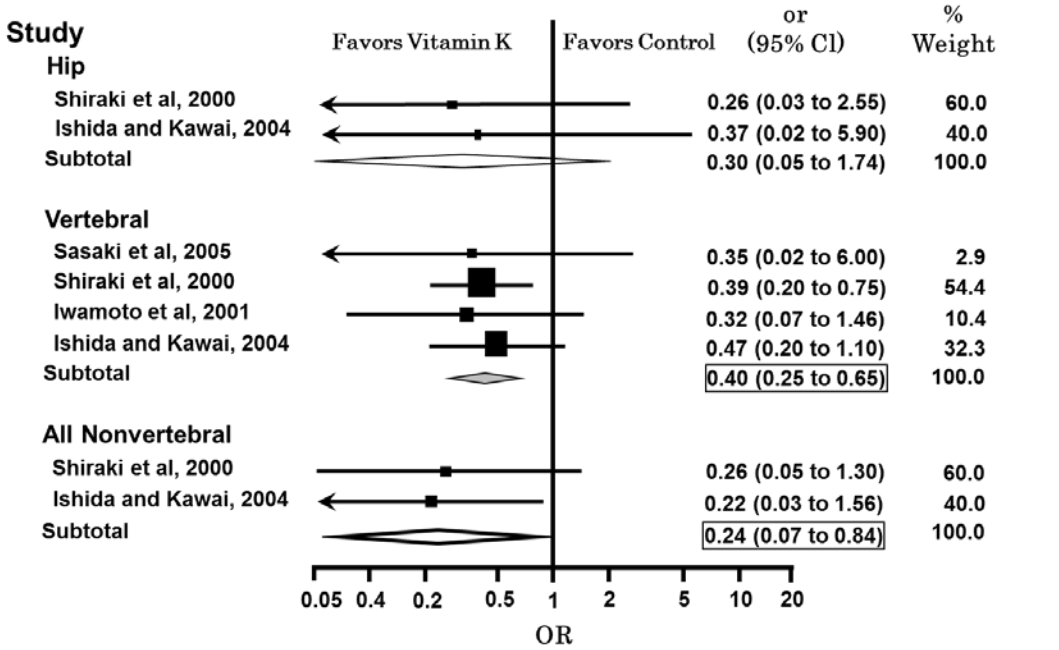


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Figure 10 New paradigm of vitamin K action in bone

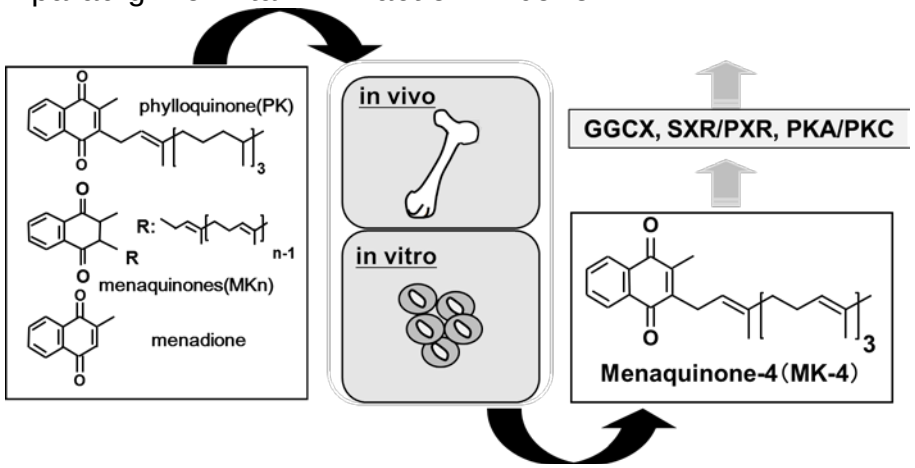
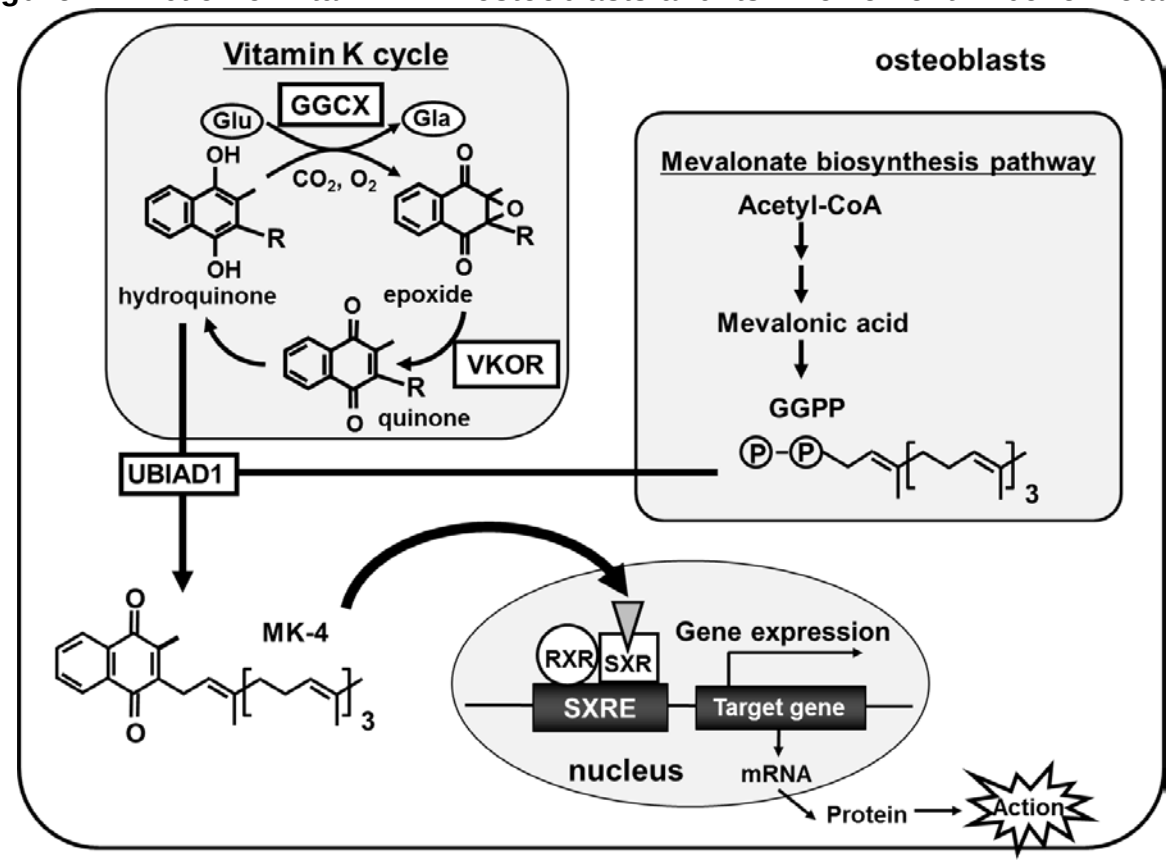


Figure 11 Action of vitamin K in osteoblasts and its involvement in bone metabolism



3. Effects of nutrition intervention on the mortality rate and the rate of complications after the occurrence of fracture in elderly people

There is a systematic review regarding the effects of nutrition intervention on the mortality rate and the rate of complications after the occurrence of fracture in elderly people[35]. The inpatients after the occurrence of femoral neck fracture or the patients in rehabilitation were the main candidates. They took orally either energy, proteins, vitamins, or minerals independently or complexly by either the intestinal tract or the intravenous route, and evaluations of the probability of survival, the period of treatment and rehabilitation, a physical function state, the necessity for care, QOL, the degree of fracture healed, etc. at the time of the end of intervention and during the medical treatment. As a result, although the supplementation of the multi-nutrients containing tablets reduced neither mortality rate nor a rate of complications independently, it was likely to be effective in reducing occurrence of undesirable phenomenon. Moreover, although the supplementation of proteins alone did not reduce mortality rate, similar effects as those observed in the case of the supplementation of the multi-nutrients containing tablets was observed. On the other hand, the supplementation of water-soluble vitamins and vitamin D alone reduced neither mortality rate nor a rate of complications. Therefore, although nutrition therapy is surely important as a basic medical treatment for reducing the mortality rate or the rate of complications after fracture generating, more therapeutic effects should not be expected.

In recent years, risk factors for osteoporosis have been identified and early diagnosis for osteoporosis became available, and consequently medication and surgical cure for osteoporosis have developed. However, it is still difficult to cure osteoporosis completely and the situation that the patients are

continuously exposed to the danger of new fracture generating is not change. Despite fall prevention is a very important issue, the concern of patients or doctors is considered not to be so high. From now on, much more development of this field will be expected.

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Chapter 5.

Nutrition and the Aging Brain

Brain Aging

Matteo Cesari*

Cognitive health is constituted by a complex combination of mental processes including the ability to learn new things, executive functioning, perception, intuition, judgement, language, and remembering. Adequate cognitive function is crucial for successful aging, and often determines the difference between dependence and independent living (1, 2).

It has been demonstrated that cognitive function slightly declines with aging. However, as reported by Wilson and colleagues using data from the Religious Order Study (3), the worsening of cognition primarily reflects person-specific factors rather than the existence of an inevitable developmental process. In fact, when the same group of Authors explored the longitudinal modifications of cognitive function of older persons, different trajectories of changes were isolated (4). In fact, while some older persons experience rapid and dramatic modifications of cognition, others follow less evident (if any) changes over time. This means that, although the aging process is conceptually the same for every individual, its manifestations are very heterogeneous from subject to subject.

The aging process assumes different features even under the same pathological condition. In other words, it is impossible to delineate a unique model of (brain) aging. In a recent review paper, Daffner and colleagues (5) proposed different curves describing the "brain aging" phenomenon in a simple and schematic manner:

1) Normal aging. Persons in this group present a relatively stable brain aging characterized by a minimal cognitive loss over time, without reaching the clinically relevant threshold of dementia;
2) Pathological aging. Subjects in this group present a steeper decline of cognitive function with aging compared to the previous group. Their curves get closer to the clinical threshold of dementia and may even pass it at (more or less) advanced age. In pathological brain aging, the curves themselves may start at a baseline lower level of cognition (closer to the disease threshold).

The difference between the "normal aging" and the "pathological aging" trajectories is mainly due to endogenous and exogenous insults occurring throughout life to our organism. These insults include clinical conditions which have the potentiality to reduce the cognitive reserves and the information processing capacity (e.g., cardiovascular disease, respiratory disease…). It is also noteworthy that the description of an aging trajectory is closely related to the instruments used to measure it. For example, it is evident that different curves of aging (sometimes presenting a linear trend, sometimes showing an exponential modification) will be described when analysing different aspects of the same cognitive ability and/or when different tools are adopted (6).

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* M.D., Ph.D. Institut du Vieillissement, Université de Toulouse, Toulouse, France

Mild cognitive impairment is defined as a cognitive decline that is severe enough to be noticeable to others and that shows up on neuropsychological testing, but it is not severe enough to interfere with daily life. It is highly frequent in the elderly (about 10-20% in persons aged 65 years and older) and considered as an intermediate condition between normality and dementia. It is associated with an increased short-term risk of developing dementia, although many cases remain stable over years (7).

Differently, dementia represents a group of progressive and irreversible diseases. Dementia might have a degenerative (e.g., Alzheimer's disease, Parkinson's disease, dementia with Lewy's bodies, front temporal dementia) or a vascular pathogenesis. Unfortunately, to date, there is no definitive treatment for dementia. Interestingly, neurodegenerative and vascular features are often simultaneously present in older patients, so that a unique pathological mechanism is difficult to currently be indicated (7).

Why is it important to discuss about "pathological" brain aging?

First of all, the threshold distinguishing what is "normal" from what is "pathological" is often arguable in this field, and (as for any other clinical condition) purely based on arbitrary decisions. Therefore, exploring age-related processes should always include the simultaneous evaluation of what is currently considered "normal" as well as what is considered "pathological".

Second, the demographic explosion occurring worldwide, especially for the oldest age groups, is creating a dramatic scenario for public health services. The progressive aging of our societies has dramatically increased the overall healthcare costs and reduced the working part of the populations. The higher prevalence of older persons also means a higher (relative and absolute) number of persons at risk of cognitive impairment and dementia. Looking at recent estimates, it has been calculated that the 24.3 millions of global patients with dementia in 2000 will become almost 4-fold more (i.e., 81.1 millions) in 2040. Some world areas, such as India and South-East Asia or China and Western Pacific, even present more dramatic trajectories (8). For this reasons, it is important to extend our knowledge on (brain) aging process and cognitive disorders to develop and put in place preventive and therapeutical interventions aimed at reducing such scenario.

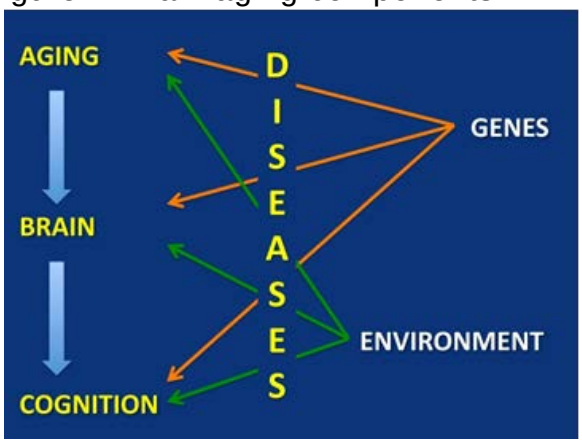
The most common type of dementia in older persons is represented by Alzheimer's disease. It is macroscopically defined by a shrinking of the overall brain volume (especially in some areas, such as the hippocampus), and the presence of brain beta-amyloid plaques and neurofibrillary tangles at microscopic level. Epidemiologic data clearly show that Alzheimer's disease (which we may consider as a paradigmatical model of pathological brain aging) is steadily and linearly increasing over time (9). However, the population is not exposed to the risk of developing Alzheimer's disease in the same way (10). In fact, after age of 70 years old, persons present an exponential increase of developing the disease. Such dramatically higher risk at advanced age has been documented in both men and women, and consistently confirmed in multiple cohort studies. This may indicate that even small improvements in the prevention and/or treatment of Alzheimer's disease will provide extremely relevant benefits to public health. It has been estimated that the gain of just one year of delay in the onset of the disease will reduce by almost 12 million the number of Alzheimer's disease patients in 2050 (with consequent major reductions in healthcare costs) (11). In fact, since the neurodegenerative disease is occurring at advanced age, a reduction of its incidence in older persons will mean that many elders will never experience such burdening clinical condition, maybe because dying for other causes. From a public health perspective, taking into account the high costs associated with cognitive disorders, this may signify having many more resources available to be

implemented in other research and healthcare activities.

If prevention of cognitive decline is important, we need to understand the underlying mechanisms determining it, so that specific actions might be put in place. Unfortunately, to date, we have multiple hypotheses potentially explaining the brain aging process, without the possibility to only focus on a single mechanism (12). For example, genetic (e.g., APOE4), toxic (e.g., environmental exposure to specific molecules), inflammatory (e.g., cytokines), vascular (e.g., hypertension, smoking, obesity), psychosocial (e.g., education, diet, physical activity, social interaction), and/or oxidative (e.g., dietary antioxidants, vitamin B12) factors have all shown to be able to positively or negative affect the aging process of the brain.

Interestingly, some signalling pathways influencing the global aging process have also been found at the basis of the age-related modification of the brain (13). For example, caloric restriction represents a major (and maybe the most promising) anti-aging intervention. It is able to reduce oxidative damage, improve mitochondrial function, and facilitate the clearance of damaged proteins. Such positive actions are naturally exerted also at the brain level. Thus, it is not particularly surprising that individuals undergoing to caloric restriction interventions present cognitive improvements (especially on memory) compared to controls (14). Moreover, such clinical benefits are associated with positive biological modifications on insulin sensitivity and inflammatory status (14). Although the benefits at multiple levels from caloric restriction do not allow a clear definition of the specific pathway targeted by the anti-aging intervention, these (preliminary) data still support the multidimensional phenotype of (brain) aging. In other words, it is impossible to understand whether the cognitive improvement is due to the higher insulin sensitivity and/or the lower inflammation and/or to any other factor not considered in the study. We can just say that the brain aging is part of a global phenomenon and shares important features and mechanisms with it.

Figure 1 Brain aging components



The more or less enhanced age-related modifications of the brain determine its functioning (i.e., cognition). This pathway (Figure 1) (15) is largely dependent on:

1) Genes. The concept that our genetic background describes our (brain) aging trajectories is well-established. For example, women are more exposed to pathological brain aging than men (due to the longer lifespan? To the loss of the estrogens neuroprotection?). Although a clear and direct transmission of the disease is not yet identified, it is evident that relatives of patients have higher risk of experiencing the disease than subjects with no history of cognitive disorders in their family. Moreover, specific genetic mutations (e.g., APOE4, presenilin-1, presenilin-2, amyloid precursor protein) have been identified and characterize specific forms of Alzheimer's disease.
2) Environment. Exogenous stressors acting throughout life on out organism may be responsible for delaying or accelerating the (brain) aging process. For example, poor diet (high in fat and calories and low in omega-3) and unhealthy lifestyle (low physical and mental activity, smoking, and alcohol

consumption) have shown to be associated with increased risk of pathological brain aging. Education has also been associated with brain aging although data are quite controversial on this topic. In fact, it seems that low education may delay the onset of the disease (perhaps through the activation of a higher reserve of cognitive compensatory mechanisms), but not extend the survival.

3) Diseases. Clinical conditions are defined by our knowledge and the accuracy of the diagnostic instruments. In other words, diseases are the result of our ability to understand the interaction of genetic patterns and environment. It is out of doubt that clinical conditions represent major determinants of our cognitive function. Mainly every disease is able to (directly or indirectly) affect brain aging. Briefly, here are listed the main conditions which are more relevantly associated with cognitive impairment:
   - **Depression**. Although it is not yet clear the cause-effect relationship between depression and cognitive impairment, their association is very well established. We have not yet understood whether depression causes cognitive impairment (i.e., a depressed individual may avoid social interaction and present an unhealthy lifestyle posing him/her at risk of dementia) or the opposite (a person becomes depressed because feeling the early signs of cognitive decline) (16). Whichever is the direction of the association, it is evident that depression and cognitive impairment share common pathophysiological basis in the increased concentrations of circulating corticosteroids and hippocampus atrophy.
   - **Cardiovascular disease**. Very recently, Jefferson and colleagues have demonstrated that measures of cardiovascular function are closely related to structural (i.e., brain volume) (17) and functional (i.e., memory) (18) measures of brain aging.
   - **Respiratory disease**. Respiratory function is able to significantly determine the ability to remain cognitively intact. For example, patients with chronic obstructive pulmonary disease (a very frequent respiratory condition at advanced age) present an increased risk of cognitive impairment. Such higher risk of developing dementia has been explained by a wide spectrum of mechanisms (including endothelial abnormalities, inflammation, atherosclerosis, oxidative damage, hypoxia) responsible for neuronal damage and dysfunction (19).
   - **Sleep disorders**. With aging, the quality and efficacy of sleep gradually declines (20, 21). Older persons present more fragmentised and superficial sleep patterns than younger adults (22). As clearly demonstrated by Oosterman and colleagues (23), cognitive performance is closely and inversely related to fragmentation of the rest-activity rhythm.
   - **Oxidative damage**. Oxidative damage represents the cornerstone of one of the most accepted theories of aging. Studies have clearly shown that oxidative damage is inversely associated with mitochondrial function, and this latter is positively associated with cognitive performance (24). Obviously, if oxidative damage is at the basis of the aging process, its detrimental effects need to be evident even in the age-related brain modifications.
   - **Hormonal abnormalities**. With aging, the feedback regulating the glucocorticoid secretion becomes less effective, leading to a condition of constant stress (13). This is confirmed by studies showing an association between poor cognitive function and a flattening of the cortisol circadian rhythm (25), and the stressed biological phe-

notype of older persons (also in terms of cognitive function) (26).

- **Malnutrition**. Nutrition may significantly affect the brain aging. Besides of deficiencies in common micronutrients able to determine the onset of cognitive impairment (e.g., vitamin B12, folates, vitamin D), a fat-brain and a gut-brain axes have been proposed. The former is based on the evidence showing the key role played by leptin on memory. The latter is focused on the relationship existing between ghrelin and hippocampus in regulating cognitive functions (27).
- **Obesity**. Just recently, Cao and colleagues (28) published results from an interesting study in mice showing that animals exposed to a physically and cognitively active environment developed a lean phenotype and resistance to obesity. Interestingly, these beneficial effects were mediated by a morphological and functional modification of adipose tissue. In fact, animals in the active group experienced the "browning" of their white adipose tissue, meaning an improved thermogenesis and insulin sensitivity.

To date, we are unaware of interventions which clearly delay the brain aging through a direct effect. In a recent review, Desai and colleagues (7) provided the following checklist (specifically composed for clinical use) to promote healthy brain aging:

1. Smoking cessation
2. Physical activity
3. Healthy nutrition (Mediterranean diet)
4. Challenging and creative leisure activities
5. Promote emotional resilience
6. Active and socially integrated lifestyle
7. Optimal daily sleep
8. Reduce risk of head injury
9. Reduce exposure to hazardous substances
10. Moderate alcohol consumption
11. Healthy weight
12. Optimal blood pressure control
13. Optimal control of dyslipidemia
14. Optimal control of blood sugar /diabetes
15. Discuss alternative treatments
16. Secondary prevention of stroke

As evident, this is a very general list of common sense and well-established beneficial healthy behaviours. It might implicitly demonstrate our lack of specific resources to target (brain) aging. On the other hand, it might be possible that to delay a so complex and dynamic mechanism as the aging process, a single intervention might not be sufficient, especially if to be put in place at advanced age (when chronic and long-standing vicious cycles are already established). In other words, it might be necessary to implement multidomain interventions rather than actions on specific and single causal mechanisms to counteract the effects of aging. Interestingly, this approach is very similar to what has been proposed to prevent and treat a major geriatric syndrome: frailty. Frailty is defined as a biologic syndrome of decreased reserve and resistance to stressors, resulting from cumulative declines across multiple physiologic systems and causing vulnerability to adverse outcomes (29). As for cognitive aging, frailty does not follow a unique trajectory, but is very person-specific and influenced by endogenous and exogenous factors (30). With this in mind, we may speculate that the heterogeneous process of (brain) aging has a very clinical relevance in frailty, which automatically translates in the need of adopting comprehensive instruments to measure it (31) and multidomain interventions to counteract its development (32). In this context, it is paradigmatical our experience of the Multidomain Alzheimer Preventive Trial (MAPT) (33) enrolling 1,600 older persons with self-reported memory complains and implementing a multidomain intervention (based on nutritional supplementation, cognitive training, and physical performance) with the aim of

preventing the onset of Alzheimer's disease. The study is currently ongoing and hopefully we will soon have the preliminary data to confirm (or confute) our hypotheses.

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Higher-Brain Network involved in Mastication

Yuji Masuda*

1. Control of mastication

Mastication is sequential action to make a bolus with chewing food. It is known that brain stem including the central pattern generator and the motorneurons of muscles in oro-facial region is most important to control the masticatory movement basically. However mastication is the oro-facial motor function, and is also required for sensory inputs from oral region. During mastication, various sensory inputs related to food (e.g. taste, consistency etc.) are delivered to brain. When mastication is thought to be a part of the nutrition process, mastication is only the animal function whereas the most processes are vegetative function. It is thought that the higher brain is needed for the control of the masticatory sequence. In fact, it is reported that the blood flow to the higher brain is increased and many higher-brain areas are activated during chewing. In the higher brain, it is suggested that cerebral cortex (Masuda et al, 2002) and the basal ganglia (Masuda et al, 2001) are involved in the control of mastication. This paper focuses on the network involved in mastication in the high brain.

2. Network involved in mastication in the high brain

2.1 Two cortical areas involved in mastication

In the cerebral cortex, two areas that induce the oro-facial movements by electrical stimulation have been found (Penfield and Rasmussen, 1950, Lund et al, 1984). One is the cortical masticatory area (CMA), to which the repetitive electrical stimulation induces the rhythmical jaw movements like masticatory movements, and the other is the primary motor cortex face area (face-MI), to which short-train stimulation induces the muscle twitch in the oro-facial region.

We investigated the location of two areas in the cortical area 0-5 mm anterior and 2-6 mm lateral to the bregma in the guinea pig, based on the evoked jaw movement by stimulation. The CMA was defined as the area to which the repetitive electrical stimulation (30 Hz, duration 0.2 ms, 6 s) induces the rhythmical jaw movements, and the face-MI was the area to which short-train stimulation (500 Hz, duration 0.3 ms, 16 ms) induces the muscle twitch in the oro-facial region. The CMA was located caudal and lateral to the face-MI with overlap between two areas. The rhythmical jaw movements evoked by the CMA is classified in two patterns, one is simple vertical jaw movement, and the other is the chewing-like pattern which is very similar to natural chewing movements. Chewing movements were complex movements composed of three phases during a chewing cycle. The area inducing chewing-like pattern was located in the most lateral area that we investigated, and in granular cortex cytoarchitecturally. The granular cortex has the clear granular layer, and receives the sensory inputs from thalamus.

* Department of Oral and Maxillofacial Biology, Graduate School of Dentistry, Matsumoto Dental University.

2.2 Cortico-cortical projection between two areas

First, projection between the CMA and the Face-MI was investigated using neuronal tract-tracing methods. When anterograde tracer [Biotinylated Dextran Amine (BDA)] was injected in the area inducing chewing-like patterns in the CMA, labeled terminals were found in the Face-MI. Retrograde tracer [Fluorogold (FG)] injection to the Face-MI caused finding of labeled cells in the area inducing chewing-like patterns. This result indicated the projection from the area inducing chewing-like patterns in the CMA to the face-MI. Second, spatial spread of excitation by stimulation to the CMA was examined by the voltage-sensitive dye. When stimulation gave the site inducing chewing-like pattern, excitation was found the area 3-5 mm anterior and 1-4 mm lateral to the bregma. This means that the excitatory connection from the area inducing chewing-like patterns to the face-MI. Moreover, the effects of inactivation of the face-MI on evoked rhythmic jaw movements were investigated. When the muscimol (agonist of GABAA receptor) was injected to the face-MI, the latency of the rhythmic jaw movements evoked by stimulation to the area inducing chewing-like patterns in the CMA was prolonged. The rhythm and pattern of evoked jaw movements were not changed by muscimol injection to the Face-MI.

These results suggest that the excitatory projection from the area inducing chewing-like patterns in the CMA to the face-MI may play an important role on the elicitation of the chewing-like movement.

2.3 Cortico-thalamic connection from two areas

Cortico-thalamic connections from two areas were investigated using neuronal tract-tracing methods.

After the BDA injection to the area inducing chewing-like pattern, a large number of anterogradely BDA-labeled axon fibers and terminals were found to be located in the ventral posteromedial nucleus (VPM) of the thalamus. After the FG injection, a large number of retrogradely FG-labeled cells were found in the medial part of the VPM, which appeared to correspond to an area containing a large number of BDA-labeled axon fibers and terminals after BDA injection. This area had reciprocal connections with the VPM of the thalamus. It is suggested that the area inducing chewing-like pattern received a lot of sensory inputs from the oro-facial region because the VPM is regarded as the relay nucleus of sensory inputs from trigeminal nerve. On the other hand, the face-MI had reciprocal connections with the ventral lateral nucleus (VL) or the ventral anterior nucleus (VA) of thalamus. The VL and VA, which were called motor thalamus, received the inputs from the cerebellum or basal ganglia.

Figure 1 Schema of two cortical areas involved in mastication in the guinea pig

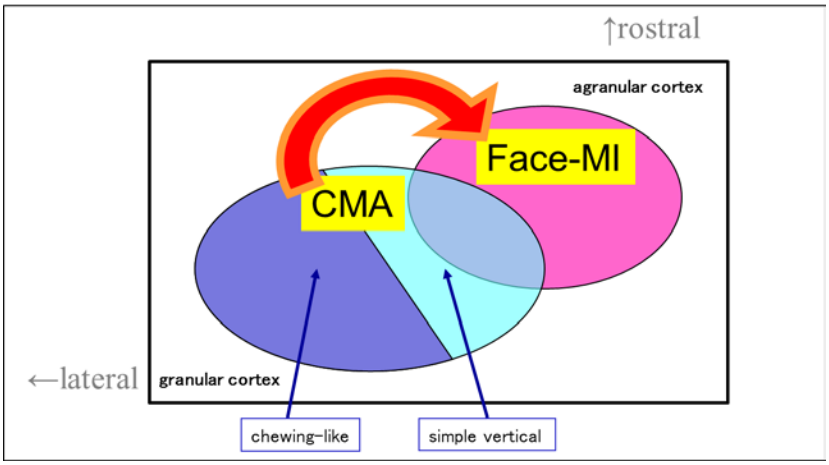
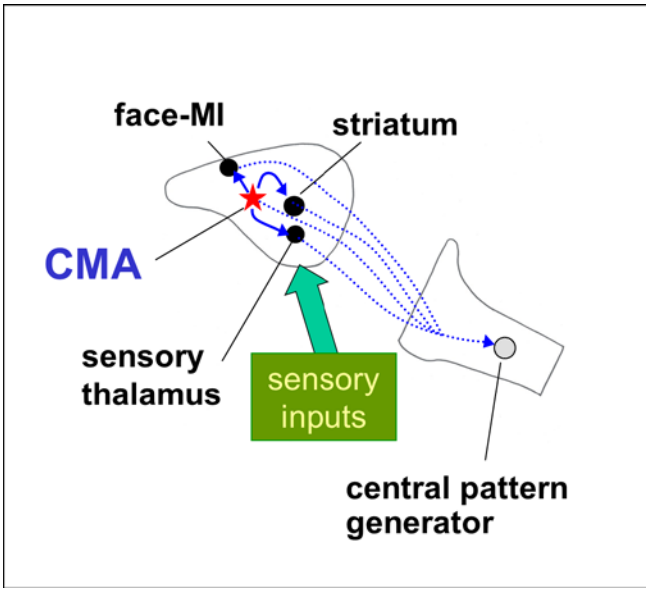


Figure 2 Schema of connection from the cortical masticatory area to brain



3. Summary

It has been considered that neurons in the CMA, which project to brainstem activate the brainstem network when electrical stimulation causes the chewing like movements. However, from the investigation of the characteristics of the CMA that induce the chewing-like movements, it is known that this area have a network in the higher brain such as the cortico-cortical projection and the cortico-basal ganglia loop. Those networks in the higher brain might play an important role on the control of masticatory movements. And, because the activity of the CMA can be elicited by oral sensation, it is suggested that the higher-brain network is necessary for masticatory accomplishment on the basis of sensory inputs from the oro-facial region.

It is found that aging cause the hypofunction of the higher brain. In order to maintain the masticatory function, these networks of higher brain, discussed here, might complement each other. It is thought that these networks could be activated by sensory inputs from the oro-facial region.

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Flavor Preferences Across the Lifespan: Birth to Aging

Gary K. Beauchamp* and Beverly J. Cowart*

The chemical senses are critical in guiding the most important decision people make every day of their lives – whether to take into our bodies a potential nutrient or to reject a potential poison. A fuller understanding of how flavor perception develops and changes throughout the life span helps in identifying strategies to facilitate healthy eating. Here an overview of research conducted mainly by my colleagues and myself at the Monell Center on developmental changes in flavor perception and preference across the life span is provided.

All three of the chemical senses are functional prior to birth but they exhibit differential developmental trajectories during early life. For example, the perception and preference for sweets is innately organized and is evident prior to birth. Preferences for umami tastes are also evident at a very early age. In contrast, the perception and preference for salt matures well after birth. Perception and preferences for the olfactory components of flavor likely also develop after birth and there is little convincing evidence for innate preferences; learning seems to be most important. Young children tend to exhibit preferences for higher concentrations of positive taste substances such as sweets and salt than do adults. The factors underlying these high preferences are not fully understood but probably involve age related differences in nutritional needs. They may also reflect differences in early exposures to these tastes.

As individuals age, the flavor senses, like all senses, exhibit gradual declines in sensitivity but the degree of decline differs greatly across the senses. In general, the sense of smell exhibits the most striking decline in sensitivity but even in these senses there is some evidence that declines are not uniform; sensitivity to some odors may decline at a greater rate than others. For taste, the declines are more modest and this sense remains remarkably robust into old age. The differential decline in the flavor senses has important implications for how food perception changes in elderly individuals. The relative flavor balance of foods changes with the olfactory components becoming less easy to distinguish relative to the taste components. This may help account for a decline in pleasure obtained from eating that has been often reported in older individuals.

The final section of this paper briefly discusses the importance of pleasure in motivating food intake and the potential role for flavor "exercise" in older individuals to maintain function and enhance eating pleasure in the elderly.

* Both Ph.D., Monell Chemical Senses Center 3500 Market Street, Philadelphia, PA 19104-3308 USA

1. Introduction

Until very recently in evolutionary time, the two most important feeding considerations for humans were first to get sufficient nutrients, particularly calories, protein, and minerals, to survive and second to make sure that one did not consume harmful substances (poisons) which are often found in plants. In marked contrast, many humans in the developed world now have a very different problem – how to limit over consumption of calorie-rich, sodium-rich foods and under consumption of nutrient-rich vegetables and fruits. But while this is true for young people and most adults, it still remains a problem for many older individuals to consume sufficient nutrients for good health. Thus we wish to understand the major factors that contribute optimal nutrition across the lifespan in order to develop strategies to reduce excess intake of some foods while enhancing intake of others, depending on life stage.

A central factor regulating food choice and intake is the flavor of the food. Two of the flavor senses, taste and smell (we do not discuss here the third chemical sense that contributes to flavor – chemical irritation or chemesthesis – due largely to the lack of developmental information on this sense) evolved to provide crucial information on the nutritional value of a food and to motivate consumption or rejection. Thus a fuller understanding of how flavor perception develops and changes throughout the life span will help in identifying approaches to facilitate healthy eating. Indeed, we argue here that comparisons between the very young person and the older person can provide important insights into the special problems of aging, flavor perception, food choice and food intake.

It is important in what follows to recognize that smell and taste are separate and distinct anatomical and physiological systems. They have different receptor types and peripheral neural pathways. In air breathing animals, they generally respond to different classes of molecules: For taste, non-volatile, easily water soluble molecules such as sugars, salts and acids, amino acids and small peptides, and bitter alkaloids; for smell, small volatile molecules that can partition in to the nasal mucus. However, central nervous system responses to many smell and taste stimuli overlap. Thus, when food is eaten—simultaneously releasing molecules that stimulate taste receptors in the oral cavity and other molecules that flow through the nasopharynx to the nose and stimulate olfactory receptors—it is perceptually very difficult to distinguish which components of the resulting flavor perception are smells and which are tastes.

Here we provide an overview of research, much of it conducted by our colleagues and ourselves at the Monell Center, on developmental changes in flavor perception and preference early in life and as a consequence of aging. Thus the main focus is on the two ends of the age spectrum – early childhood and the elderly where two complimentary issues relevant to insuring good nutrition and health eating are evident. These two issues are the importance of the hedonic component of the flavor senses and the second is the importance of learning in establishing sensitivity and preference for some flavor components.

What follows is divided into three sections. The first outlines flavor perception early in life with an emphasis on infancy. This portion of this review is updated from Beauchamp & Mennella (2009)[1]. The second section provides a brief summary of some of the many published studies on the other end of the age spectrum: changes in flavor perception in the older individual. This section is modified from Cowart (2010)[2]. Finally, the last brief section emphasizes important areas for future research on development and aging of flavor perception.

2. Flavor Perception and Preference in Infancy and Childhood

Scientists at Monell have been investigating the determinants of flavor perception

and preference in a program of research that has spanned more than 40 years. This research has encompassed many different investigations but generally has focused on the interacting role of genetic and experiential influences in the human fetus, the infant and the child.

(1) Taste

Liking for taste stimuli has a large genetic component.[3] For example, sweet foods are innately preferred by humans, most likely because sweetness reflects the presence of caloric sugars in plants. In this regard, Humans resemble most other herbivores and omnivores studied. Indeed a preference seems to be characteristic of all but those animals that consume exclusively animals as food (strict carnivores). Infants and children tend to prefer higher concentrations of sweeteners in foods and beverages compared with adults. This age related differences have been attributed to the greater need for calories during the rapid growth phase of infancy and childhood. In spite of this strong innate component for sweet liking, preferences can also be modified by experience.[4] For umami substances such as glutamate, IMP and GMP, an innate component to liking is also likely although there is considerably less research than has been conducted for sweet.[4]

Most investigators believe that bitter taste functions to signal the presence of potentially toxic compounds and hence substances that are bitter are generally disliked and avoided and this avoidance is particularly striking in infants and young children. Here, this powerful rejection of bitter tasting foods has been attributed to the particular danger the young may risk in eating food that contains a poison Even for adults, the majority of bitter foods and beverages that are liked are those with positive pharmacological properties (e.g. coffee, tea and alcoholic beverages) and learning seems to be required to establish acceptability.[5]

Salt taste (primarily NaCl) preference continues to mature after birth, and preferences are not generally observed until around 4 months of age. By 2 years of age, children's preferences for high salt in foods are even greater than they are for adults.[4;6] Prenatal developmental events may modify the infant's and child's preferences for salty tastes. For example, severe maternal emesis can have an enduring influence on response of offspring to salty taste.[7;8;9] We recently reported[10] that several some measures of salty taste preference are inversely related to birth weight over the first 4 years of life. Early experiences with salty foods may also play an important role in the early development of salt taste liking.[11] These observations are potentially important in light of concerns that excess salt intake plays a role in the development and maintenance of hypertension. Thus, one set of factors predisposing people to high salt intake may be the heightened preferences that have an innate component that is importantly modified by experiences both before and soon after birth.

To summarize early development of taste, there is a strong innate component to the hedonic tone of taste qualities. Sweet, umami and probably salt liking are innately positive and predispose to intake whereas bitter and perhaps sour are innately negative and predispose to rejection. These innate responses can be modified by experiences, both prenatal and postnatal, but they predominate in control of intake in infants and children.

(2) **Smell**.

In contrast to taste, preferences for flavor compounds detected by the sense of smell are much more highly influenced by learning. Moreover, learning early in life, even prior to birth, is particularly important.[3;12]

(3) **Prenatal Learning**.

One route by which early exposure to flavor compounds detected by olfactory receptors is through the amniotic fluid. During the last trimester of gestation, the olfactory

system is sufficiently mature to detect flavor compounds. These flavor compounds can enter this fluid as a consequence of uptake from food flavors the mother consumes.[13] Experiences with such flavors lead to heightened preferences for them shortly at birth[14;15] and at weaning.[16] In one experimental study, mothers of infants were randomly assigned to drink carrot juice during the last trimester of pregnancy. These infants responded more positively to carrot-flavored enjoyed cereal when it was first presented at 6 months of age compared with infants whose mothers did not consume carrot juice.[16] Thus, experiences with food flavors consumed by the pregnant woman led to greater acceptance and enjoyment of foods with these flavors during weaning and likely beyond.

(4) Postnatal Learning: Infant Formulas.

Standard commercial cow milk (CM) formulas differ enormously in flavor from those made with hydrolyzed casein (HC formulas). These HC formulas are used primarily for formula-fed infants who cannot tolerate intact cow milk proteins. The flavor of HC formulas is extremely unpleasant to those unfamiliar with them, having a bitter and sour taste, and a nauseating smell and aftertaste.[17] Infants less than 3 – 4 months of age with no prior exposure to HC formulas readily accept them and appear to actually like them.[18;19] In contrast, infants over 5 – 6 months of age with no prior exposure to HC formulas strongly reject them and, based on facial expression analyses, dislike them intensely.[18;19] However, 5-6-month old (or older) infants who were exposed to these HC formulas during the first few months of life appear to relish them upon formal acceptance testing.[20]

These findings demonstrate a dramatic sensitive learning period in the first several months of life during which unpalatable flavors (to those not familiar with them) can be rendered palatable. During HC formula exposure early in life, infants form a flavor image which, when later matched, elicits pleasure and drives intake. Additional evidence suggests that these experiences with HC extend to similar flavors in other foods[21] and are long-lived.[22;23]

(5) Postnatal Learning: Human Milk.

Studies of HC formulas likely have direct relevance to more normal feeding with human milk.. Human milk is flavored by the foods and beverages ingested or inhaled by the mother.[24;25;26;27] Thus, the breast fed infant is exposed to the changing flavor world of the mother. These experiences influence the infants' subsequent liking and acceptance of these flavors in foods[16;28;29] as has been reported for other mammals[30;31;32] and as was found in our studies of HC formulas. Breast milk serves as a 'bridge' between the pre natal experiences with flavors in amniotic fluid to those in solid foods at weaning and beyond. Breast feeding (unlike formula feeding) provides the infant with the potential for a rich source of varying flavor experiences.

These early sensory experiences with flavors in breast milk impact on food choice. For example, the study referred to earlier[16] on carrot flavor exposure in utero also included a group of breast feeding infants whose mothers consumed carrot juice during the first 3 months of the infants' life. This post natal exposure to carrot flavors in breast milk enhanced the infants' responses to carrot flavor when they were tested at weaning just as in utero experience did. In another study, breast fed infants were more accepting of peaches than formula-fed infants, as determined by intake, rate of consumption and facial expressions. This enhanced acceptance of fruit was likely due to more exposure to fruit flavors since their mothers ate more fruits during lactation.[29] Variety in flavor experiences during early ontogeny may be the reason that, compared to formula fed infants, breastfed infants are less picky[33] and are more willing to try new foods.[34]

In summary, there are few data indicating an innate preference for the volatiles (odors) in food. Instead, early experiences with flavor compounds carried in amniotic fluid and in milk (human or formula) establish later flavor and food preferences. These preferences and the hedonic quality of food flavors may persist throughout an individual's life.

3. Flavor Perception and Preference in the Aging Individual

It is assumed that with age all sensory systems decline. Is this true? And if it is, is there anything that can be done to slow or prevent declines? These questions are particularly relevant for the flavor senses (taste and smell) since they impact directly on food acceptance and intake. Unlike the problems with over consumption of nutrients for children and younger adults, there are major concerns of under consumption of nutrients by older individuals.[35] In fact, research at Monell and other institutions has shown that these two aspects of food flavors are not equally affected by aging: diminutions in smell sensitivity are more pronounced than diminutions in taste sensitivity.

(1) Taste.

Taste receptors (responding to sweet, salty, sour, bitter and umami, or "savory", stimuli) may be damaged by chemicals they are designed to detect, as well as by viruses, bacteria and fungi that may inhabit the oral cavity. However, since these receptors are scattered over a large portion of the top and sides of the tongue, as well as being found on the soft palate, esophagus, pharynx and epiglottis, and since their responses are transmitted to the brain by multiple branches of three cranial nerves, they are protected against extensive damage. And although there is evidence of reductions in the number of taste buds with age and reduced neural responsiveness to tastes, this sense is relatively unaffected by age in comparison to olfaction (see below).

Some age-related declines in both taste threshold sensitivity and the perceived intensity of suprathreshold tastes are have been observed. However, these declines are often found to be quality- and, in the case of bitter, compound-specific, and they are not always observed in both threshold and suprathreshold measures within a quality.[36;37] Indeed, the majority of recent studies have found little or no age-related decline in sensitivity to sweetness (primarily by sucrose), and declines in sensitivity to salty, sour and bitter tastes, at either threshold or suprathreshold levels, are modest relative to those observed in smell. Because umami has only recently gained wide acceptance as a basic taste, very few studies have examined how it is impacted by age although there is little reason to expect that it will show marked declines. There are also few large, lifespan studies of taste function that report some declines but these appear to be significant only in the 7th and 8th decades.

All of the above findings are based on presenting taste stimuli to the whole mouth. Several studies, however, suggest the elderly are particularly prone to spotty losses of function affecting circumscribed areas of the tongue.[38;39] Although this generally has little impact on the normal experiences of taste in the whole mouth, as other areas appear to compensate, it may render elderly individuals more vulnerable to taste dysfunctions. Consistent with this, research in our taste & smell clinic has found that elderly patients ($\geq$ 65 years of age) are significantly more likely than young or middle-aged patients to complain of the persistent unpleasant or phantom taste sensations.[40] This raises the concern that as our population ages, the prevalence of taste problems may increase as well, and these problems can be particularly significant because they have a greater negative impact on food intake than do smell problems.[41]

(2) Olfaction.

Olfactory receptors are more restricted in location compared to taste receptors and

are more easily exposed to potentially damaging toxins and microbes. And although olfactory receptor neurons are highly unusual in that they are capable of regeneration throughout life, this is a complex process requiring reinnervation of the olfactory bulb (the first brain relay in the olfactory pathway), and it is often imperfect. Consequently, degeneration of the olfactory neural tissue, and patchy replacement by respiratory tissue, is seen even in young adults and becomes more pronounced with aging. All of this is consistent with the observed major losses of olfactory functioning with age.

Olfactory function, most often assessed using tests of threshold sensitivity or of the ability to identify more concentrated odors (e.g., is this orange, licorice, grass or banana?), has uniformly been reported to show significant decline with age. Typically, these declines begin in the 7th or 8th decade of life although they can begin much earlier. A recent population-based epidemiological study of olfactory impairment in the U.S. found the prevalence of loss to be only 6% among fifty-year-olds, but 17% among sixty-year-olds, 29% among seventy-years olds, and over 60% in those 80-97 years of age.[42] While age-related olfactory loss appears to develop gradually and is rarely complete, except in extreme old age, it is often of sufficient magnitude to render older people vulnerable to chemical hazards such as gas leaks[43] and to greatly diminish olfactory food flavor perception,[44] reducing food enjoyment.

Although virtually all researchers agree that average olfactory sensitivity declines with age, the uniformity of that decline, both across different odors and across individuals is not clear. Results from the National Geographic Smell Survey conducted by Monell scientists suggest perception of some odors declines earlier than others. Notably, the ability to detect a mixture of mercaptans (sulfur compounds added to natural gas as a warning agent) began to decline abruptly in the fifth decade, whereas for several other odors included in the survey (eugenol, isoamyl acetate and rose), declines in performance first became evident in the sixth decade and did not accelerate steeply until the eighth.[45] In addition, at the individual level, extreme differences among elderly subjects in olfactory abilities have frequently been noted, with some older individuals performing as well as the average young person. However, specific genetic, medical and/or environmental factors that underlie this variation have not been identified.

In summary, as individuals age, the flavor senses, like all senses, exhibit gradual declines in sensitivity but the degree of decline differs greatly across the senses. In general, the sense of smell exhibits the most striking decline in sensitivity but even in these senses there is some evidence that declines are not uniform; sensitivity to some odors may decline at a greater rate than others. For taste, the declines are more modest and this sense remains remarkably robust into old age. The differential decline in the flavor senses has important implications for how food perception changes in elderly individuals. The relative flavor balance of foods changes with the olfactory components becoming less easy to distinguish relative to the taste components. This may help account for a decline in pleasure obtained from eating that has been often reported in older individuals.

4. Flavor Perception Across the Age Span: Lessons Learned and Future Potential

In this final section we briefly consider lessons learned from a lifespan consideration of the flavor senses and focus in particular what can be gleaned from comparisons between infancy and old age that may prove useful for maximizing the nutritional and sensory health of the elderly. Here we focus particularly on two aspects of the flavor senses, their particular and powerful role in hedonics or pleasure and the im-

portance of sensory experience in establishing and maintaining optimal functioning.

First consider pleasure. As we have emphasized above in the section on early flavor development, the senses of taste and smell are characterized by their hedonic tone. Often you may not be able to name the identity of a flavor but you seldom have any trouble determining whether you like it. The innate underpinnings of liking and disliking for taste qualities has made stimuli such as sweet, salt, umami (all generally positive) and bitter (generally negative) powerful reinforcers. The fact that taste function is well preserved into old age is a gift – it means that foods and eating can remain a source of profound pleasure.

Next, consider learning and experience. As we indicated in the section of flavor development, early experiences can have a profound effect on the flavor senses. In particular, experiences with volatile flavors transmitted from mother to fetus via amniotic fluid and mother to infant via her milk can establish preferences and aversions that may last a lifetime. But what about flavor experience in the elderly? A series of animal model studies as well as a few human studies strongly suggest that adult experiences can have profound influences on the flavor senses.[46] Underlying such effects may be the nature of these senses where receptor cells for both taste and smell routinely regenerate – something that does not commonly occur for the other sensory systems. This raises the intriguing possibility that later experience, a sort of exercise of the flavor senses, might help forestall loss, particularly for olfaction where as we saw losses are most common. In a recent pilot study, Beverly Cowart and Marcia Pelchat at Monelll provide the first tentative indication that such a phenomenon may occur. If further work, which is ongoing, supports this, it would have important implications for how we approach the aging of the flavor senses. Taste function is already well maintained into old age; wouldn't it be wonderful if smell could also be preserved via a form of exercise?

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Chapter 6.

Scientific Investigation by Genomics

Biochemical investigation and gene expression analysis of the immunostimulatory functions of an edible Salacia extract in rat small intestine

Yuriko Oda*

Roots and bark from plants belonging to genus *Salacia* of the family *Hippocrateaceae* (*Salacia reticulata*, *Salacia oblonga*, etc.) have been used for traditional Ayurvedic medicine particular by for the treatment of diabetes. In our study, we evaluated the gene expression profiles in the small-intestinal epithelium of rats given a *Salacia* plant extract in order to gain insight into its effects on the small intestine. In detail, DNA microarray analysis was performed to evaluate the gene expression profiles in the rat ileal epithelium. The intestinal bacterial flora was also studied using T-RFLP (Nagashima method) in these rats. Expressions of many immune-related genes, especially Th1-related genes associated with cell-mediated immunity, were found to increase in the small-intestinal epithelium and the intestinal bacterial flora became similar to those in the case with *Salacia* plant extract administration. Our study thus revealed that *Salacia* plant extract exerts bioregulatory functions by boosting intestinal immunity.

1. Introduction

Various components including salacinol, kotalanol, mangiferin and catechins have been identified in extracts from plants belonging to the genus *Salacia* (*Salacia reticulata*, *Salacia oblonga*, etc.), while there are many components that remain to be identified (*1*). Salacinol and kotalanol extracted from *Salacia* plants have been shown to exert an inhibitory effect *in vitro* on both α-glucosidase activity and blood glucose elevation in glucose-loaded rats (*2*). The small intestine, where α-glucosidase is secreted, is a very important organ with critical functions such as foreign substance exclusion, nutrient uptake, and immunity. Thus, *Salacia* plant extract is considered to exert useful effects in the small intestine, although the underlying mechanisms remain to be precisely elucidated. This extract has also been demonstrated to improve symptoms in diabetic patients as well as diabetic mouse models (*3*). Some compounds found in *Salacia* plant extract, such as catechins and mangiferin, are known to have an anti-obesity effect (*4*), and an increasing number of novel findings about *Salacia* plants have been reported in recent years (*5*,*6*). However, little is known about other functions of *Salacia* plant extract or the mechanisms underlying its biological effects. In particular, synergies among multiple components of the extract function to be clarified.

In this study, gene expression profiles were analyzed by the microarray technique and intestinal bacterial flora were examined by the T-RFLP method in rats administered *Salacia* plant extract, in order to elucidate the physiological functions of the extract.

* Pharmaceutical & Healthcare Research Laboratories, Fujifilm Corporation.

2. Materials and Methods

Preparation of *Salacia* plant extract powder

Bark and roots of *Salacia reticulata* grown in Sri Lanka were collected, dried and chipped. After being dried completely, the chipped material was extracted in hot water for an hour. The extract was filtered to remove the chips, cooled, and then pulverized by spray-drying (ADL-310, Yamato Science Co., Ltd., Tokyo Japan), prior to storage at 4°C.

Animals

Six-week-old male rats, Sprague Dawley® (SD), were purchased (CLEA Japan, Inc., Shizuoka, Japan), then quarantined and conditioned for 1 week prior to the experiment. The animals were housed under the following conditions: room temperature, 23°C ± 2°C; relative humidity, 50% ± 10%; ventilation frequency, 15 times/hour; artificial lighting, 12 hours/day. The animals had free access to an irradiation-sterilized solid diet, CRF-1 (Oriental Yeast Co., Ltd., Tokyo, Japan), and also to filtered (50 μm and 5 μm filters) and UV-sterilized tap water (compliant with the water quality standard of the Water Supply Law). After quarantining and conditioning for 1 week, the rats were divided into groups of 10 animals each. *Salacia* plant extract powder was dissolved in Water for Injection (Otsuka Pharmaceutical Co., Ltd., Tokyo, Japan) at a concentration of 80 mg/mL, and administered intragastrically to the rats via gavage using a metal feeding needle at a dose of 20 mg/kg (as *Salacia* extract powder); The Water for Injection alone was given to control group rats. Administration was repeated once daily for 13 weeks. The animals were fasted for 16 hours starting on the evening of the final administration. The following morning, blood specimens were collected under sodium pentobarbital anesthesia, and the animals were euthanized by exsanguination. Autopsy was conducted for weight measurement and examination of individual organs. Epithelial cells were separated from the excised ileum and preserved in ISOGEN (NIPPON GENE Co., Ltd., Tokyo, Japan). Fecal specimens were collected from the lower large intestine and stored frozen with solid carbon dioxide.

Blood was collected from the ventral prostate at autopsy in tubes containing the anticoagulant EDTA-2K, and subjected to biochemical testing.

The blood parameters examined included: white blood cell count (WBC), red blood cell count (RBC), hemoglobin (HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), platelet count (PLT), reticulocyte ratio (Reti), prothrombin time (PT), activated partial thromboplastin time (APTT), total protein (TP), albumin concentration (Alb), A/G ratio, triglyceride (TG), total cholesterol (T-CHO), blood urea nitrogen (BUN), creatinine (Cre), calcium (Ca), inorganic phosphorus (IP), AST activity (AST), ALT activity (ALT), CPK activity (CPK), total bilirubin (T-Bil), sodium (Na), potassium (K), and chloride (Cl). The WBC, RBC, HGB, HCT, MCV, MCH, MCHC, PLT, and Reti values were analyzed using the integrated blood test system XT-2000iV (Sysmex Co., Ltd., Hyogo, Japan). PT and APTT were measured in an automated blood coagulation and fibrinolysis analyzer, SAT compact (Roche Diagnostics K.K. Tokyo, Japan), and the TP, ALB, A/G ratio, Glu, TG, T-CHO, BUN, Cre, Ca, IP, AST, ALT, GGT, ALP, CPK, T-Bil, Na, K, and Cl values were analyzed using an automated blood biochemical analyzer, H 7070 (Hitachi Ltd, Tokyo, Japan).

Statistical analysis was performed to detect between-group differences in body weights and organ weights (absolute weights and relative weights) and also in blood biochemical data. The F-test for homogeneity of variance was first performed, and the statistical significance of differences was evaluated by Student's *t*-test. Tissue specimens were preserved and fixed in 10% neutral buffered formalin, thin sections were prepared, stained with hematoxylin-eosin (HE staining) and observed under an optical microscope. Differences in general condition, morphopathological test results and histopathological test results were not statistically analyzed.

All of the animal experiments were approved by the Animal Care and Use Committee for Fujifilm.

RNA Extraction and DNA Microarray Analysis

Total RNA was extracted from preserved rat ileal cells according to the ISOGEN standard method, and purified using the RNeasy Mini Kit (QIAGEN, Hilden, Germany). Four animals closest in body weight to the mean in each *Salacia* extract-treated group and the control group were selected for further analysis. cDNA was prepared from total RNA extracted from the ileal specimens of the selected animals, cRNA was synthesized and labeled, and the labeled cRNA was fragmented using the Affymetrix kit in accordance with the manufacturer's protocol. RNA quality was assessed using an Agilent 2100 bioanalyzer (Agilent Technologies Japan, Ltd., Tokyo, Japan) to confirm sufficient cRNA elongation. Fragmented cRNA was hybridized with the GeneChip Rat Genome 230 2.0 Array (Affymetrix Inc., CA, USA) at 45°C for 16 hours using Hybridization Oven 640 (Affymetrix Inc., CA, USA), washed and stained using GeneChip® Fluidics Station 450, and the gene expression levels were analyzed using the GeneChip® Scanner 3000. The obtained data were summarized by the Distribution Free Weighted (DFW) method using R version 2.7.2 and Bioconductor version 2.2, and probes with a false detection rate (FDR) of < 0.05 were extracted as a probe set showing significant expression variation (*7-11*).

The extracted probe set was categorized by biological function using Gene Ontology (BiNGO 2.3 (cytoscape 2.6)) (http://www.psb.ugent.be/cbd/papers/BiNGO/index.htm) as the reference, and presented in a hierarchical structure by function (*12, 13*).

Intestinal Bacterial Flora Analysis (T-RFLP method)

Analysis of intestinal bacterial flora using rat fecal specimens was outsourced to Techno-Suruga Laboratory Co., Ltd. (Shizuoka, Japan), where the T-RFLP method was used (*14*). In brief, frozen fecal specimens were suspended in GTC buffer (100mM Tris-HCl [pH 9.0], 40mM Tris-EDTA [pH 8.0], 4M guanidine thiocyanate). Fecal solids in the suspension were broken down using the FastPrep FP100A Instrument (MP Biomedicals, CA, USA) with zirconia beads at 5 m/s for 5 min. DNA was extracted from a 100 μL suspension using an automatic nucleic acid extractor (Precision System Science, Chiba, Japan). GC series Genomic DNA whole blood (Precision System Science) was used as the reagent for the automatic nucleic acid extraction. The PCR primer for 516F labeling was switched from HEX described in the reference to FAM. PCR products were purified using a MultiScreen PCRμ-96 plate (Millipore, Billerica, MA, USA).

Fragment analysis was performed with the ABI PRISM 3130xl genetic analyzer (Applied Biosystems, CA, USA) using the DNA analysis software Gene Mapper (Applied Biosystems). MapMarkerR X-Rhodamine Labeled 50–1000bp (BIOVENTURES, TN, USA) was used as the size standard marker. Bacterial flora were compared by hierarchical cluster analysis (using pvclust) of the ratio of the peak area to the total area of each OUT (operational taxonomic unit).

3. Results

Biochemical Test Values

No significant between-group differences were observed in body weight (541.2 ± 47.8 g vs. 578.2 ± 76.0 g) or blood parameters (WBC, RBC, HGB, HCT, MCV, MCH, MCHC, PLT, Reti, TP, ALB, A/G ratio, Glu, TG, T-CHO, BUN, Cre, Ca, IP, AST, ALT, GGT, ALP, CPK, T-Bil, Na, K, Cl). All individual animals were subjected to autopsy, and the brain, pituitary gland, thymus, lungs, liver, kidneys, spleen, heart, adrenal glands, testes, epididymis, seminal vesicles, and prostate gland ventral lobe were weighed; the liver was excised and fixed in 10% neutral buffered formalin, and sections prepared from the fixed specimens were stained with hematoxylin-eosin (HE stain) and examined by optical microscopy. The above analyses revealed no pathological changes indicative of toxicity under the experimental conditions employed.

Microarray Analysis

After confirming the absence of findings indicative of toxicity, the four animals closest in body weight to the mean in each group were

selected for microarray analysis. Based on the results of the microarray analysis, a set of 237 genes showing increased expression and a set of 111 genes showing decreased expression in the *Salacia* group, as compared with the control group, were extracted.

Genes Showing up-regulation

Genes identified as showing increased expressions were mostly those related to oligopeptide transport, defense responses, responses to nutrient levels, and antigen processing and presentation of peptide or polysaccharide antigens via major histocompatibility complex (MHC) class antigens. Closer examination revealed enhanced expressions of many of defense-related genes, including immune-related genes and genes involved in transport and metabolism (Figure 1).

Among the MHC class II-related genes involved in antigen recognition, elevated expressions were observed for the genes for cathepsin E (Ctse), RT1 class II locus Ba (RT1-Ba), HLA class II histocompatibility antigen, and DM beta chain precursor (MHC class II antigen DMb, Hla-dmb)*(15)*.

Defense (immune)-related genes showing increased expressions included the genes for tumor necrosis factor alpha (Tnfα), clusterin (Clu), chemokine (C-C motif) ligand 5 (Ccl5, Rantes), adenosine deaminase (Ada), apolipoprotein A-IV (Apoa5), chemokine (C-X-C motif) receptor 4 (Cxcr4), apolipoprotein H (Apoh), membrane-spanning 4-domains subfamily A member 1 (Ms4a1), dipeptidyl-peptidase 4 (Dpp4, Cd26), protein tyrosine phosphatase (Ptprc, Cd45), T cell receptor beta locus (Tcrb) and apoptotic peptidase activating factor 1 (Apaf1). Expressions of the genes for 3-hydroxy-3-methylglutaryl-CoA synthase 2(Hmgcs2), an enzyme involved in cholesterol and ketone body metabolism, and for solute carrier family 15 member 1 (Slc15a1), an oligopeptide transporter in the transport system (Table 1), were also increased.

Figure 1 Significant gene ontology categories (P < 0.001) were extracted from 237 genes showing increased expression.

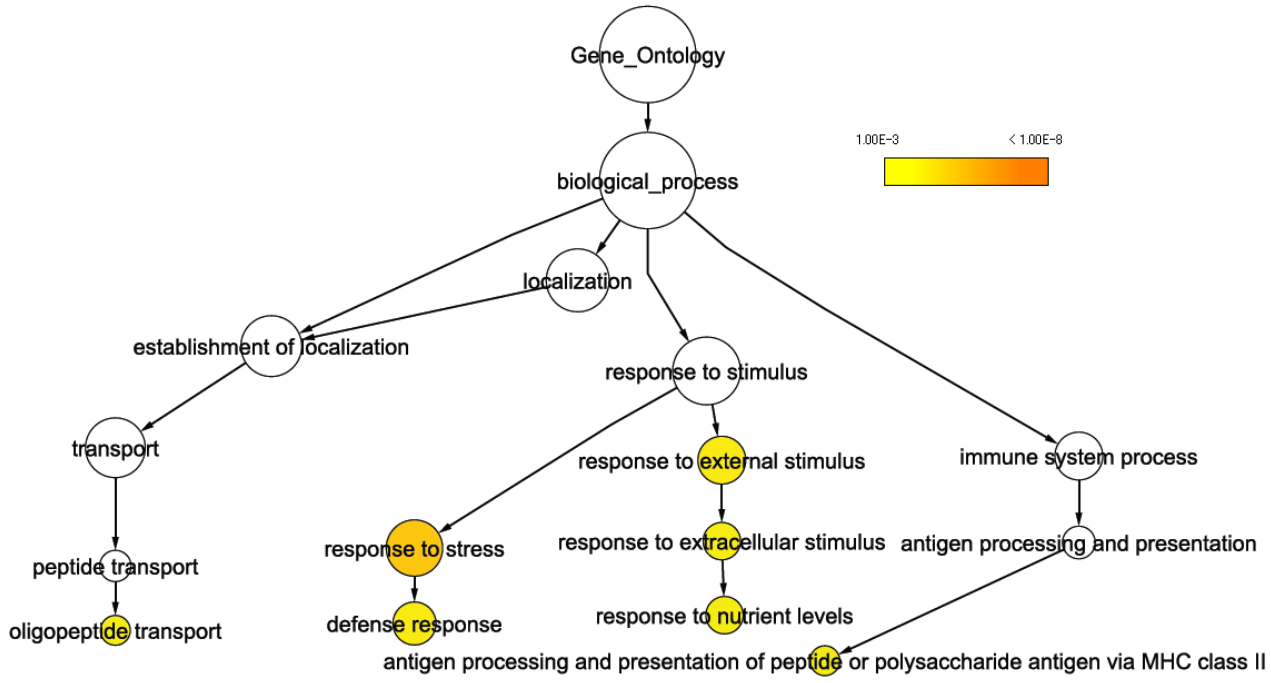


Table 1 Genes showing increased expression (P < 0.001, Gene ontology categories extracted using BiNGO)

【response to stress】		
Gene name	Definition	UniGene ID
Tnf	tumor necrosis factor	Rn.2275
Aldob	aldolase B	Rn.98207
Clu	clusterin	Rn.1780
Atp6v1g2	ATPase	Rn.158467
Abhd2	abhydrolase domain containing 2	Rn.136611
Sfn	stratifin	Rn.145079
RT1-Ba	RT1 class II, locus Ba	Rn.25717
Ccl5	chemokine (C-C motif) ligand 5,	Rn.8019
Hla-dmb	major histocompatibility complex	Rn.5892
Ada	adenosine deaminase	Rn.12689
RT1-Aw2	RT1 class Ib, locus Aw2	Rn.40130
Apoa4	apolipoprotein A-IV	Rn.15739
RatNP-3b	rat neutrophil peptide-1	Rn.114810
Alb	albumin	Rn.202968
Cxcr4	chemokine (C-X-C motif) receptor 4	Rn.44431
Gsn	gelsolin	Rn.103770
Apoh	apolipoprotein H (beta-2-glycoprotein I)	Rn.1824
Ms4a1	membrane-spanning 4-domains	Rn.16385
Creb3l3	cAMP responsive element binding protein 3-like 3	Rn.20059
Cfd	complement factor D (adipsin)	Rn.16172
Dpp4	dipeptidyl-peptidase 4 (CD26)	Rn.91364
Car3	carbonic anhydrase 3	Rn.1647
Ptprc	protein tyrosine phosphatase	Rn.90166
Bmp2	bone morphogenetic protein 2	Rn.90931
Si	sucrase-isomaltase	Rn.10057
Ephx2	Epoxide hydrolase2	Rn.54495
Tcrb	T cell receptor beta locus	Rn.34871
Adipoq	adiponectin, C1Q and collagen domain containing	Rn.24299
Defa-rs1	defensin alpha-related sequence 1	Rn.122020
Cyp4f5	cytochrome P450 4F5	Rn.10171
Abcc2	ATP-binding cassette	Rn.10265
Apaf1	apoptotic peptidase activating factor 1	Rn.64522
Prnp	prion protein	Rn.3936
Ta4sf4	transmembrane 4 L six family member 4	Rn.13425
【response to external stimulus】		
Gene name	Definition	UniGene ID
Suox	sulfite oxidase	Rn.25720
Bmp2	bone morphogenetic protein 2	Rn.90931
Tnf	tumor necrosis factor	Rn.2275
Si	sucrase-isomaltase	Rn.10057
Clu	clusterin	Rn.1780
Aldob	aldolase B	Rn.98207
Ephx2	Epoxide hydrolase2	Rn.54495
Abhd2	abhydrolase domain containing 2	Rn.136611
Ccl5	chemokine (C-C motif) ligand 5,	Rn.8019
Adipoq	adiponectin, C1Q and collagen domain containing	Rn.24299
Ada	adenosine deaminase	Rn.12689
Apoa4	apolipoprotein A-IV	Rn.15739
Coro1a	coronin	Rn.6990
Apoa1	apolipoprotein A-I	Rn.10308
Cyp4f5	cytochrome P450 4F5	Rn.10171
Hmgcs2	3-hydroxy-3-methylglutaryl-Coenzyme A synthase 2	Rn.29594
Gsn	gelsolin	Rn.103770
Alb	albumin	Rn.202968
Ms4a1	membrane-spanning 4-domains	Rn.16385
Apoh	apolipoprotein H (beta-2-glycoprotein I)	Rn.1824
Cfd	complement factor D (adipsin)	Rn.16172
Tm4sf4	transmembrane 4 L six family member 4	Rn.13425
Smpd2	sphingomyelin phosphodiesterase 2	Rn.18572

To be continued

Continued

【defense response】		
Gene name	Definition	UniGene ID
Ptprc	protein tyrosine phosphatase	Rn.90166
Bmp2	bone morphogenetic protein 2	Rn.90931
Tnf	tumor necrosis factor	Rn.2275
Ephx2	Epoxide hydrolase2	Rn.54495
Tcrb	T cell receptor beta locus	Rn.34871
RT1-Ba	RT1 class II, locus Ba	Rn.25717
Ccl5	chemokine (C-C motif) ligand 5,	Rn.8019
Hla-dmb	major histocompatibility complex	Rn.5892
Defa-rs1	defensin alpha-related sequence 1	Rn.122020
Ratnp-3b	rat neutrophil peptide-1	Rn.114810
Apoa4	apolipoprotein A-IV	Rn.15739
Cyp4f5	cytochrome P450 4F5	Rn.10171
Ms4a1	membrane-spanning 4-domains	Rn.16385
Apaf1	apoptotic peptidase activating factor 1	Rn.64522
Cfd	complement factor D (adipsin)	Rn.16172
【response to nutrient levels】		
Gene name	Definition	UniGene ID
Apoa4	apolipoprotein A-IV	Rn.15739
Suox	sulfite oxidase	Rn.25720
Bmp2	bone morphogenetic protein 2	Rn.90931
Apoa1	apolipoprotein A-I	Rn.10308
Hmgcs2	3-hydroxy-3-methylglutaryl-Coenzyme A synthase 2	Rn.29594
Gsn	gelsolin	Rn.103770
Alb	albumin	Rn.202968
Si	sucrase-isomaltase	Rn.10057
Aldob	aldolase B	Rn.98207
Adipoq	adiponectin, C1Q and collagen domain containing	Rn.24299
Ada	adenosine deaminase	Rn.12689
【oligopeptide transport】		
Gene name	Definition	UniGene ID
Slc15a1	solute carrier family 15 (oligopeptide transporter)	Rn.10500
RT1-Ba	RT1 class II, locus Ba	Rn.25717
Hla-dmb	major histocompatibility complex	Rn.5892
【response to extracellular stimulus】		
Gene name	Definition	UniGene ID
Apoa4	apolipoprotein A-IV	Rn.15739
Suox	sulfite oxidase	Rn.25720
Bmp2	bone morphogenetic protein 2	Rn.90931
Apoa1	apolipoprotein A-I	Rn.10308
Hmgcs2	3-hydroxy-3-methylglutaryl-Coenzyme A synthase 2	Rn.29594
Gsn	gelsolin	Rn.103770
Alb	albumin	Rn.202968
Si	sucrase-isomaltase	Rn.10057
Aldob	aldolase B	Rn.98207
Adipoq	adiponectin, C1Q and collagen domain containing	Rn.24299
Ada	adenosine deaminase	Rn.12689
【antigen processing and presentation of peptide or polysaccharide antigen via MHC class Ⅱ】		
Gene name	Definition	UniGene ID
Ctse	cathepsin E	Rn.92738
RT1-Ba	RT1 class II, locus Ba	Rn.25717
Hla-dmb	major histocompatibility complex	Rn.5892

Genes Showing down-regulation

Genes related to the urea cycle and lipid metabolic processes were identified in the group of genes showing decreased expressions. Gene expression was decreased for urea cycle-related genes, including arginase type II (Arg2), ornithine carbamoyltransferase (Otc) and carbamoyl-phosphate synthase 1 (Cps1), and for those involved in lipid transport and metabolism such as peroxiredoxin 6 (Prdx6) and peroxisome proliferator-activated receptor gamma (Pparg) (Figure 2) (Table 2).

Figure 2 Significant gene ontology categories (P < 0.001) were extracted from 113 genes showing decreased expression.

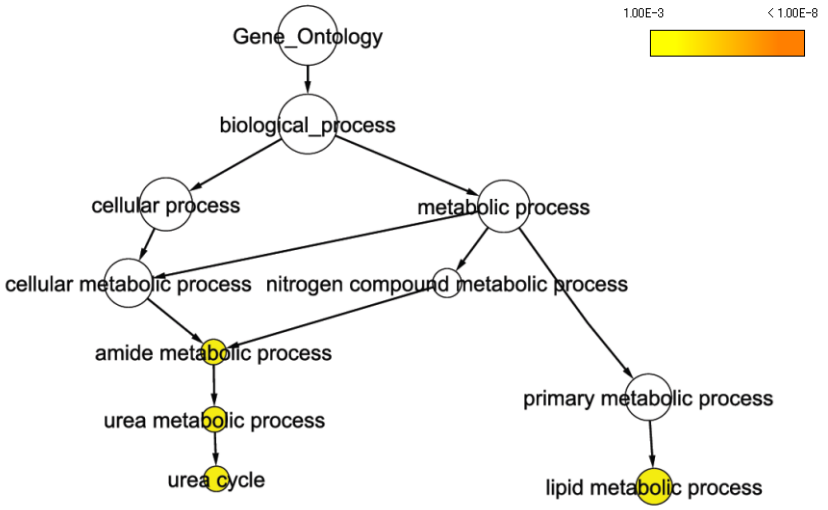


Table 2 Genes showing decreased expression (P < 0.001, Gene ontology categories extracted using BiNGO)

【urea cycle】		
Gene name	Definition	UniGene ID
Arg2	arginase	Rn.11055
Otc	ornithine carbamoyltransferase	Rn.2391
Cps1	carbamoyl-phosphate synthase 1	Rn.53968
【urea matabolic process】		
Gene name	Definition	UniGene ID
Arg2	arginase	Rn.11055
Otc	ornithine carbamoyltransferase	Rn.2391
Cps1	carbamoyl-phosphate synthase 1	Rn.53968
【amide metabolic process】		
Gene name	Definition	UniGene ID
Arg2	arginase	Rn.11055
Otc	ornithine carbamoyltransferase	Rn.2391
Cps1	carbamoyl-phosphate synthase 1	Rn.53968
【lipid metabolic process】		
Gene name	Definition	UniGene ID
Phlpb	phospholipase B	Rn.91079
Cubn	cubilin (intrinsic factor-cobalamin receptor)	Rn.3236
Hsd3b6	hydroxy-delta-5-steroid dehydrogenase, 3 beta- and steroid delt	Rn.109394
Prdx6	peroxiredoxin 6	Rn.42
Pparg	peroxisome proliferator-activated receptor gamma	Rn.23443
Hsd11b2	hydroxysteroid (11-beta) dehydrogenase 2	Rn.10186
Aldh1a7	aldehyde dehydrogenase family 1, subfamily A7	Rn.74044
Srd5a1	steroid-5-alpha-reductase	Rn.4620
Comt	catechol-O-methyltransferase	Rn.220
Pcca	propionyl Coenzyme A carboxylase	Rn.6033
Pck1	phosphoenolpyruvate carboxykinase 1	Rn.104376

Intestinal Bacterial Flora Analysis

The gene expression analysis showed that many immune-related genes were up-regulated in the ileal epithelium of animals administered *Salacia* plant extract. Therefore, analysis was also carried out to compare intestinal bacterial flora, which are considered to affect the expressions of immune-related genes in the intestine, in animals with and without *Salacia* plant extract administration. Intestinal bacterial flora were analyzed using fecal specimens collected from the lower large intestine at autopsy. Since many of the bacteria residing in the large intestine cannot be cultivated, the intestinal bacterial

flora composition was determined by the T-RFLP method, by which the actual bacterial composition can be precisely characterized in the absence of bacterial culture. Clustering was performed by R using the composition ratio of the intestinal bacterial flora, and a phylogenetic tree was constructed for similarity comparisons among the bacterial flora. The results demonstrated differences in bacterial flora between the *Salacia*-treated group and the control group (Figure 3).

Figure 3 Fecal specimens were analyzed by T-RFLP (Nagashima method) and presented as the intestinal bacterial flora composition by OTU.
□: Control, ▨: Treated * $p < 0.05$

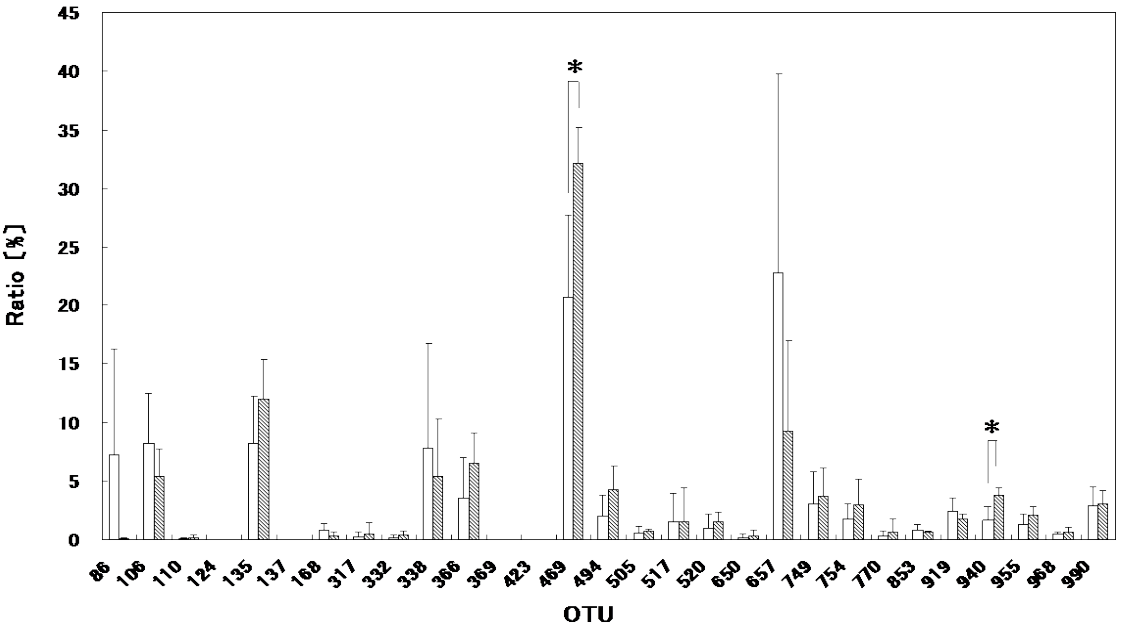
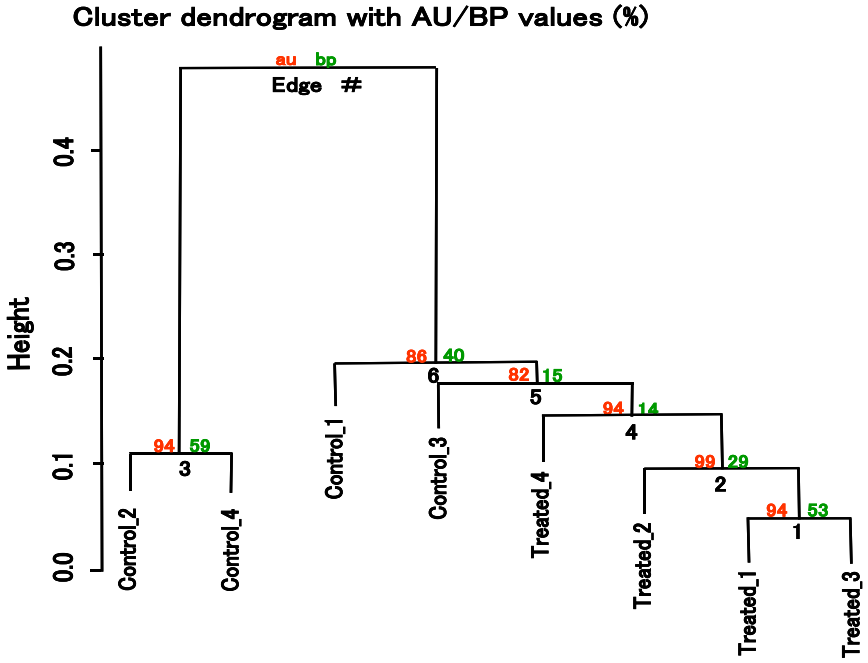


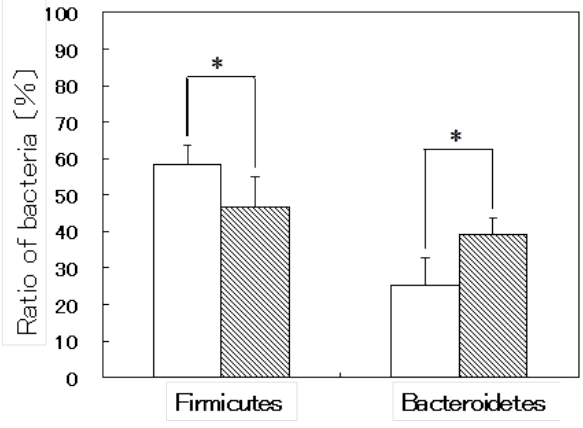
Figure 4 Cluster analysis was performed on the intestinal bacterial flora composition data determined by T-RFLP analysis (Nagashima method) to construct a phylogenetic tree. (au＝Approximately Unbiased, bp=Bootstrap Probability)



The control group showed greater individual differences in the intestinal bacterial flora and lower similarity among the bacterial species, as indicated by the wider distances on the phylogenetic tree, than the *Salacia* plant extract-treated group. In contrast, the treated group showed a higher similarity in bacterial flora among individuals and the bacterial species were positioned closer together on the phylogenetic tree, demonstrating that following administration of *Salacia* plant extract, the intestinal bacterial flora changed towards a greater degree of similarity among individuals (Figure 4).

When categorized by phylum, a significant decrease in the ratio of Firmicutes (OTU: 106, 110, 168, 332, 338, 369, 423, 494, 505, 517, 520, 650, 657, 749, 754, 919, 940, 955, 990) was observed, with a significant increase in the ratio of Bacteroidetes (OUT:366, 469, 853) in the *Salacia* plant extract-treated group (Figure 5). The above results demonstrated that the administration of *Salacia* plant extract not only induced changes in the expressions of immune-related genes, but also in the composition of the intestinal bacterial flora.

Figure 5 Proportions of Bacteroidetes and Firmicutes relative to the entire intestinal flora population in the fecesia extract administration.
□: Control, ▨ : Treated, * p <0.05, n=4



4. Discussion

Salacia plants have long been known for their various medicinal properties, and this study was undertaken to elucidate its physiological functions in the intestinal tract. The expressions of many genes in the ileal epithelium were altered after administration of *Salacia* plant extract, indicating that it exerts multiple effects in the intestine as well. In particular, altered expressions of immune-related genes were found for the first time in this study. Therefore, the following discussion focuses mainly on the immunoregulatory functions of *Salacia* plant extract.

Close examination of the results of gene expression analysis revealed that the genes showing increased expressions included many of those related to non-self recognition, immune system and host defense, especially Th1 cell-related genes. Some specific examples are: Ptprc (Cd45), considered to inhibit allergy-inducing IgE production (*16*), Th1 cell-related gene Cd26 (Dpp4), involved in cell-mediated immunity (*17*), IgG2a (*18*), which suppresses the invasion of pathogens including various bacteria and viruses, e.g., the influenza virus, and exerts an allergy-suppressive effect, and MHC class II-related genes. Based on the genes identified as showing elevated expression, a possible mechanism of action is proposed, which may operate in the vicinity of Th1 cells (*19*) (Figure 6).

Our previous study showed that *Salacia* plant extract reduces the levels of decomposition products and ammonia in the intestine. We therefore speculate that its intake reduces intestinal ammonia levels, which in turn lowers the expression levels of urea cycle-related genes (Cps1, Arg2, Otc) in the small intestinal epithelium (*20*).

In the analysis of intestinal bacterial flora, cells and their components that are considered to be closely involved in intestinal immunity, the individually varying flora tended to show similar patterns after administration of *Salacia* plant extract. Bacteroidetes bacteria, the proportion of which was found to be elevated in the treated group, are recognized for their immunostimulatory effects. It has been demonstrated that these bacteria show stronger immune-related functions than lactic acid bacteria, which have traditionally been known for their immunostimulatory effects, and that they increase the productions of IgA and cytokines in-

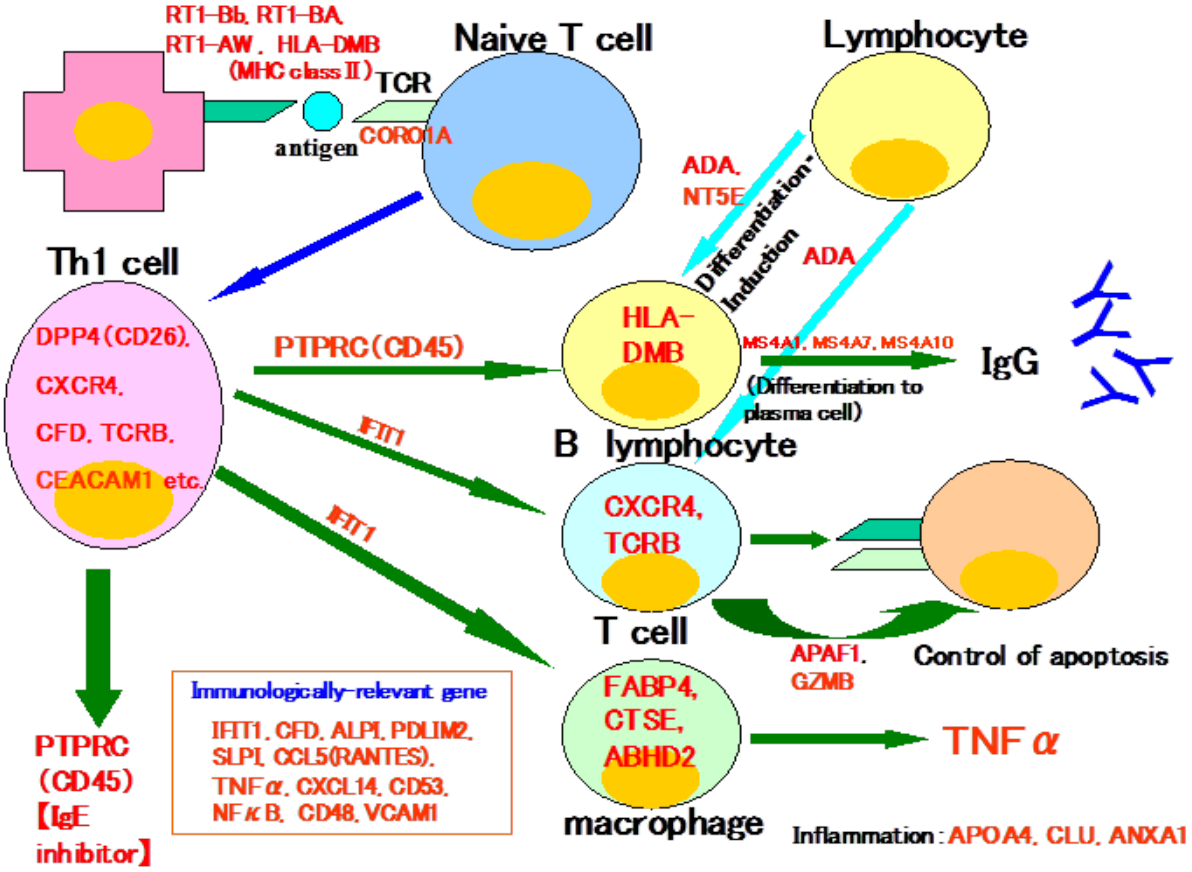
volved in host defense (*21*,*22*). In the present experiment, an especially significant increase was observed in the density of two Bacteroidetes OTUs (366 and 469), and it was found from a homology search of cloned sequences that these two are likely to contain *Bacteroides acidifaciens*, a species with a particularly strong immunostimulatory effect. Lipopolysaccharides in the cell wall of Bacteroidetes species have also been demonstrated to exert immunostimulatory effects (*23*).

The above findings may suggest that *Salacia* plant extract alters the intestinal bacterial flora and in turn stimulates the immune system in the lower small intestine. The present study also identified altered expressions of many transport- and metabolism-related genes. These genes are associated with liver function as well.

The results cannot necessarily be extrapolated to the human system, because bifidobacteria, which are abundant in humans, were not present in the rats in our experiment (*24*). However, it is very likely that in humans as well, the edible *Salacia* plant extract stimulates immune functions through alterations in the intestinal bacterial flora.

Plants belonging to the genus *Salacia* have long been used in traditional Ayurvedic medicine, but much remains to be learned about their functions. In this study, we elucidated an important aspect of the functions of *Salacia* plant extract by demonstrating its bioregulatory functions via enhanced intestinal immunity. Our findings would support the potential usefulness of this plant extract for many ailments involving intestinal immunity.

Figure 6 Possible mechanism of action speculated from the genes identified as showing increased expressions in the vicinity of Th1 cells (genes listed in Table 1 are shown in red, genes identified among the 237 genes showing increased expression but not used in the functional categorization by BiNGO are presented in black).



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Expression Change of the Insulin Signaling Pathway Related Genes in Liver of Type 2 Diabetic GK Rats by Administration of Persimmon Peel Extract

Ryoichi Izuchi*

1. Background and purposes

A persimmon (*Dyospyros kaki* Thunb.) is a fruit tree cultured not only in East Asia such as China, South Korea and Japan, but also currently in many parts of the world. It is known that the concentrations of carotenoids, polyphenols and others are higher in the peel than the pulp of the fruit. However, large quantities of the peel generated as a byproduct of processing dried persimmon are discarded without using. We have been studying the efficient use of the peel, because compounds included in the peel can contribute to human health.

The peel is tough and difficult to eat, and an odor like a dried persimmon becomes stronger by heating process. Therefore, we examined using it as extract, since it was hard to use as it is. Fat-soluble extract of persimmon peel (PPE) was prepared and analyzed for compounds. Content of β-cryptoxanthin in PPE was richly 13.4 mg/g, and quercetin (counted as aglycon), the richest polyphenol in PPE[1)] was 2.6 mg/g. It is reported that β-cryptoxanthin[2, 3)] and quercetin[4, 5)] has palliative effect on a symptoms of diabetic mellitus (DM). So, we expected prevention and improvement of DM, and investigated an effect of PPE administration.

2. Methods and results

(1) Change of plasma ALT activity and accumulation of β-cryptoxanthin in liver by administration of PPE

Goto-Kakizaki (GK) rats, lean type 2 diabetic mellitus model, were divided into normal diet (AIN-93G) group (ND) and PPE diet (PPE was added 37.3 mg/kg diet; β-cryptoxanthin, 0.5mg/kg) group (PD), and fed these diets for 12 weeks *ad libitum*. Their body weights and food intakes were measured every other day, and blood plasma and livers were collected after administration periods.

Body weights, total food intakes, and levels of glucose and insulin in plasma were not different between PD and ND. However, plasma ALT activity, hepatocyte injury marker, in PD was significantly lower than ND (Fig. 1), and β-cryptoxanthin accumulated only in the liver of PD (Fig. 2). From these results, we inferred that the state of the liver was changed and tried to clarify an effect on administration of PPE.

(2) Gene expression changes in liver by administration of PPE

It was shown using DNA microarray that 937 genes in liver of PD were up-regulated and 1263 genes were down-regulated, compared to gene expression in liver of ND. From gene-enrichment analysis of these genes using DAVID (http://david.abcc.ncifcrf.gov), genes of insulin signaling pathway associated with DM were included richly; 14 genes up-regulated and 19 genes down- regulated. Especially, glycolysis (*Gk*, *Pyk*, *Pfk*) and fatty acid synthesis (*Fas*, *Acc*) related genes and gluconeogenesis (*G6Pase*) and β-oxidation (*Cpt1*) related genes

* Toyo Institute of Food Technology.

were included in up and down regulated genes respectively. It was suggested that insulin signaling pathway was activated by PPE, because the gene expression pattern had the same tendency as that of tissue responding to insulin.

(3) Activation of insulin receptor β subunit (IRβ) by administration of PPE

To investigate whether insulin signaling pathway activated, tyrosine phosphorylation of insulin receptor β subunit (IRβ) was examined. Quantity of IRβ protein was not different between two groups, but phosphorylated-tyrosine of IRβ in PD was more major than ND (Fig.3). This result indicated that IRβ was activated by PPE. It was suggested a possibility that gene expression alterations in downstream of insulin signaling pathway arose as result of IRβ activation.

Figure 1 Difference of plasma ALT activity between two groups (n = 7/group)

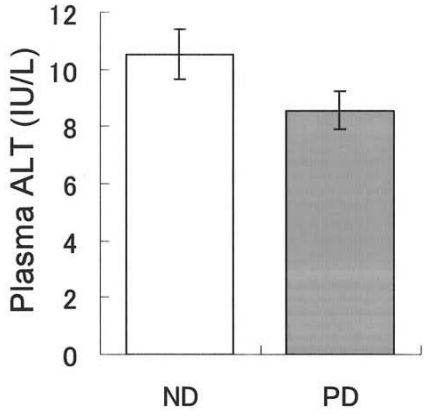


Figure 2 HPLC chromatograms of β-cryptoxanthin in the livers of two groups. Detection wavelength is 450 nm

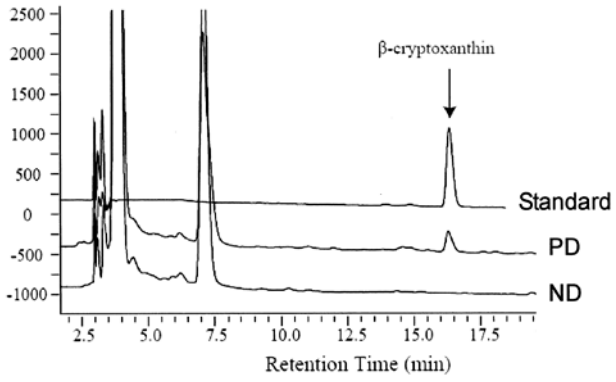


Figure 3 Change in tyrosine- phosphorylation of insulin receptor β subunit (IRβ)

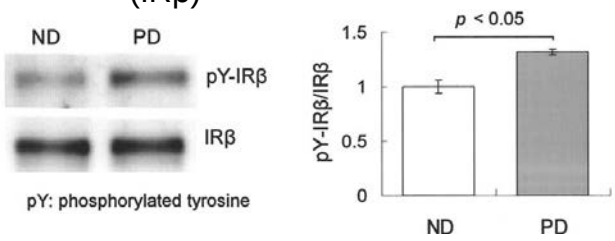


Figure 4 Difference between two groups about expression quantity of *Ptpσ* mRNA in the liver of GK rats

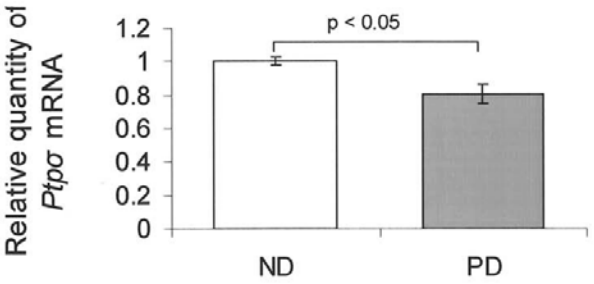
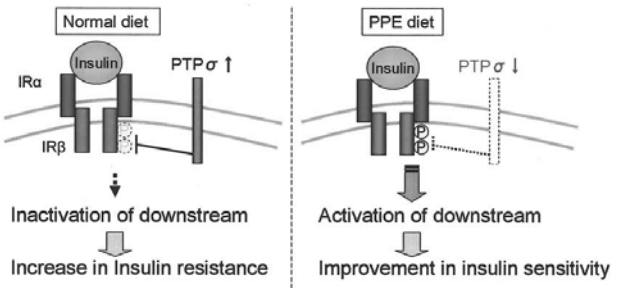


Figure 5 Hypothesis about IRβ activation in the liver of GK rat by intake of PPE.



(4) Relationship between IRβ activation and PTPσ (Protein tyrosine phosphatase sigma)

We examined how IRβ was activated by administration of PPE. It was indicated by gene expression analysis that expression of *Ptpσ* mRNA in the liver of PD decreased (Fig. 4). It is reported that PTPσ inhibit tyrosine phosphorylation of IRβ[6], the expression increase in liver of GK rat than normal rat[7], and in mouse lacking *Ptpσ* insulin sensitivity had increased [8]. We hypothesized that *Ptpσ* expression decreased in liver of PD rat would activate IRβ, and improve insulin sensitivity, and then regulate expression of the downstream factor (Fig. 5). In future, we would like to verify this hypothesis and to investigate mechanism of IRβ activation by PPE.

3. Conclusion

In the present work, we indicated the possibility that intake of PPE activated IRβ, altered expression of insulin signaling pathway related genes, and increased insulin sensitivity. Persimmon peel should be a useful food material for the prevention of DM.

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Administration of tomato modifies hepatic glucose and lipid metabolism in mice[1)]

Koichi Aizawa*

1. Introduction

Fruits and vegetables contain many beneficial nutrients and phytochemicals that are thought to protect our body against chronic diseases, such as cardiovascular disease and diabetes. Particularly, many previous reports have shown that the dietary intake of tomatoes (*Lycopersicum esculentum*) and tomato products is associated with a reduced risk of chronic diseases. Tomatoes or their components, such as a lycopene, may exert their distinct effects via different mechanisms. However, the biochemical pathways involved in these effects are largely unknown. DNA microarray technology has enabled comprehensive analysis of the expression of a large number of genes simultaneously. Therefore, we used DNA microarrays to investigate the effects of administration of tomato to normal mice on gene expression in the liver.

2. Materials and Methods

Commercially available salt-free tomato juice (Kagome Co., Ltd., Tokyo, Japan) was diluted 1:1 (v/v) with sterile water for drinking use. The resulting sample was used as the tomato beverage (TB).

Twelve specific-pathogen-free female Balb/c mice, aged 3 weeks, were divided into two groups with equal average body weight. The commercial normal chow and sterile water were given to the Control group for 6 weeks, with *ad libitum* access. For the TB group, the sterile water was replaced with the diluted tomato beverage. All animals were treated in accordance with guidelines established by the Japanese Society of Nutrition and Food Science.

Six weeks after starting the test, the mice were euthanized under diethyl ether anesthesia and their blood and liver were collected. Liver samples were frozen immediately after excision and kept at –80°C. The blood sample was used to measure the blood glucose level, plasma total cholesterol, triglyceride (TG), high-density lipoprotein-cholesterol (HDL-C), insulin and adiponectin levels, using commercial test kits. Statistical analyses were performed by ANOVA with Dunnett's multiple comparison of means test and differences were considered significant at $P < 0.05$.

For the microarray analysis, total RNA was isolated from liver samples and purified. DNA microarray analysis was performed using Affymetrix Gene Chip mouse genome 430 2.0 array for the detection of 43000 genes. The CEL files were quantified with the 'Factor Analysis for Robust Microarray Summarization (q. FARMS)' algorithm[2)] using the statistical language R and Bioconductor. Hierarchical clustering was then performed by the `pvclust()` function in R. To detect the differentially expressed genes between the control group and one of the diluted beverage groups, the Rank products method[3)] was used. To detect the over-represented Gene Ontology (GO) categories in each group of differentially expressed genes, we used DAVID, a web-accessible program.

* Ph.D., Research Institute, Kagome Co., Ltd.

3. Results and Discussion

The metabolic parameters of mice in each group are shown in **Table 1**. All mice consumed similar amounts of food together with water or beverage throughout the experimental period. At 3 weeks, there was no significant difference in body weight between each group, but the body weight at 6 weeks in the TB group was significantly lower than those in the Control group. In addition, the relative liver weight was significantly lower in the TB group. There were no significant differences in the plasma insulin or adiponectin concentrations.

To characterize the mechanism underlying the significant effect observed in the TB group, hepatic gene expression levels were analyzed using a DNA microarray. Four mice from each group, whose final body weights, relative liver weights and plasma glucose levels approximated the mean values for the six mice in each group, were selected for further DNA microarray analysis. Hierarchical clustering analysis was performed for all genes, revealing that the mice in the control and TB group separately formed apparent clusters (Data was not shown). Therefore, gene expression profiles were formed to assess the beverage-induced transcriptional changes. In this study, the genes showing a false discovery rate (FDR) of less than 0.05 between the control group and TB group were defined as genes showing biologically significant changes in expression levels. Based on this estimation, we found that the ingestion of the TB up-regulated the expression of 687 genes and down-regulated the expression of 841 genes.

Table 1 Metabolic parameters of mice given the experimental beverages[a]

	Cont	TB
beverage or water intake (g/day)	**6.59 ± 0.53**	**6.45 ± 0.40**
initial body weight[b] (g)	**10.56 ± 0.28**	**10.21 ± 0.18**
halfway body weight[c] (g)	**18.11 ± 0.21**	**17.25 ± 0.26**
final body weight[d] (g)	**20.23 ± 0.11**	**17.00 ± 0.42****
relative liver weight (g/100g of BW)	**4.75 ± 0.05**	**4.25 ± 0.09****
blood glucose (mg/dL)	**124.0 ± 9.1**	**93.8 ± 9.6**
plasma insulin (ng/mL)	**0.40± 0.22**	**0.39 ± 0.20**
plasma lipids		
total cholesterol (mg/dL)	**110.6 ± 4.0**	**119.9 ± 6.7**
triglyceride (mg/dL)	**163.7 ± 9.4**	**161.5 ± 10.4**
HDL-cholesterol (mg/dL)	**58.5 ± 2.4**	**67.8 ± 2.1**
plasma adiponectin (mg/mL)	**33.9 ± 1.2**	**35.5 ± 2.7**

[a] Values are means + SEM, n=6. * and ** indicate differences from the Control group at P <0.05 and P <0.01 by Dunnett's multiple comparison test. bInitial body weight was measured before starting dietary protocols. cHalfway body weight was measured after administering the experimental beverages for 3 weeks. dFinal body weight was measured administering the experimental beverages for 6 weeks.

Table 2 Significantly enriched GO terms found in 687 Up-regulated genes by tomato beverage ingestion (P < 0.05)

GO-ID	GO term	No. of genes	FDR-corrected *P*-value
0006457	protein folding	27	7.02E-06
0006091	generation of precursor metabolites and energy	41	2.16E-04
0006629	lipid metabolic process	43	1.11E-03
0044255	cellular lipid metabolic process	38	2.43E-03
0006638	neutral lipid metabolic process	9	1.15E-03
0046486	glycerolipid metabolic process	9	1.16E-03
0006639	acylglycerol metabolic process	9	1.15E-03
0006641	triacylglycerol metabolic process	8	1.12E-03
0006662	glycerol ether metabolic process	9	1.15E-03
0006082	organic acid metabolic process	38	1.73E-03
0019752	carboxylic acid metabolic process	37	1.20E-03
0032787	monocarboxylic acid metabolic process	22	2.70E-03
0051789	response to protein stimulus	13	1.39E-03
0006986	response to unfolded protein	13	1.39E-03
0006066	cellular alcohol metabolic process	23	2.11E-02

Table 3 Significantly enriched GO terms found in 841 Down-regulated genes by tomato beverage ingestion (P < 0.05)

GO-ID	GO term	No. of genes	FDR-corrected *P*-value
0006629	lipid metabolic process	72	5.84E-11
0008610	lipid biosynthetic process	38	1.76E-08
0006694	steroid biosynthetic process	24	4.54E-11
0016126	sterol biosynthetic process	15	8.58E-09
0006695	cholesterol biosynthetic process	12	8.36E-07
0008203	cholesterol metabolic process	19	2.61E-07
0016125	sterol metabolic process	23	5.01E-10
0008202	steroid metabolic process	33	4.86E-11
0044255	cellular lipid metabolic process	67	2.64E-11
0006066	cellular alcohol metabolic process	40	3.11E-08
0006082	organic acid metabolic process	52	2.31E-06
0019752	carboxylic acid metabolic process	52	2.35E-06
0032787	monocarboxylic acid metabolic process	32	2.49E-06
0006631	fatty acid metabolic process	23	3.59E-04
0051186	cofactor metabolic process	25	4.35E-03
0006732	coenzyme metabolic process	22	4.51E-03

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Using DAVID, the differentially expressed genes by ingestion of each beverage were classified into functional categories according to GO. The significantly enriched categories of genes that were up- or down-regulated by the administration of the TB are summarized in **Tables 2** and **3**. Accordingly, the categories of up-regulated and down-regulated genes were predominantly related to lipid and glucose metabolism in mice given the TB. Furthermore, the changes in lipid and glucose metabolism may be responsible for the decreases in body and liver weights in these mice. Therefore, we subsequently selected and categorized the genes related to glucose and lipid metabolism. The se-

lection was based on metabolic function in the gene ontology and metabolic pathway map.

The genes that were notably affected by the ingestion of TB, and those associated with glucose metabolism are listed in **Table 4**. The ingestion of the TB altered the expression of six genes related to glucose metabolism, of which two were related to glycogenesis, and were up-regulated. Two genes related to glycolysis were down-regulated, and the two genes related to gluconeogenesis were up-regulated. These changes in gene expression suggest that the ingestion of the TB enhances glycogen accumulation (**Figure 1**).

With respect to lipid metabolism, eight genes in the fatty acid synthesis pathway were down-regulated. Four genes related to fatty acid degradation were up-regulated, while three other genes were down- regulated. With respect to cholesterol synthesis, the expression of 3-hydroxy-3- methylglutaryl-coenzyme A reductase (*Hmgcr*) was up-regulated, but, down-regulation of eight genes was observed. Furthermore, six genes related to cholesterol catabolism were down-regulated (**Table 5**). These results indicate that the ingestion of the TB would decrease the biosynthesis of fatty acids and stimulate specific steps in the fatty acid oxidation pathway (**Figure 1**).

Table 4 DNA microarray data on glucose metabolism-related genes induced by tomato beverage ingestion in liver of Balb/c mice

gene name	symbol	gene-expression	false discovery rate[a]	accession no.[b]
Glycogenesis				
glucokinase	*Gck*	UP	< 0.0001	NM_010292
glycogen synthase 2	*Gys2*	UP	0.00015	NM_145572
Glycolysis				
dihydrolipoamide s-acetyltransferase (e2 component of pyruvate dehydrogenase complex)	*Dlat*	DOWN	0.00074	AV336908
pyruvate kinase liver and red blood cell	*Pklr*	DOWN	0.00133	NM_001099779, NM_013631
Gluconeogenesis				
phosphoenolpyruvate carboxykinase 1, cytosolic	*Pck1*	UP	0.00017	NM_011044
fructose bisphosphatase 1	*Fbp1*	UP	0.03393	NM_019395

[a]False discoverry rate (FDR) between the control group and the tomato beverage group. In this experiment, genes at FDR < 0.05 were defined as showing biologically significant changes in expression levels. [b]GenBank ID.

Figure 1 Summarized pathways of probable glucose and lipid metabolism in mouse liver affected by the ingestion of tomato beverage.

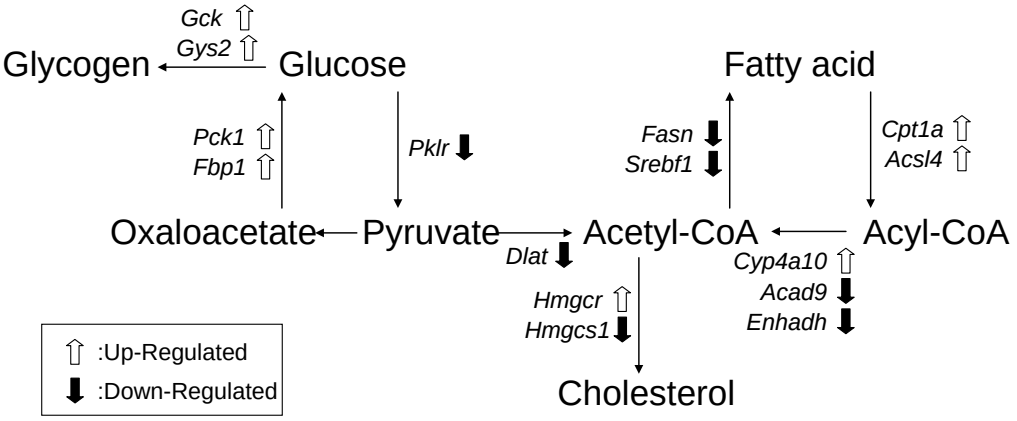

Table 5 DNA microarray data on lipid metabolism-related genes induced by tomato beverage ingestion in liver of Balb/c mice

gene name	symbol	gene-expression	false discovery rate[a]	accession no.[b]
Fatty acid synthesis				
elovl family member 5, elongation of long chain fatty acids	*Elovl5*	DOWN	< 0.0001	NM_134255
elovl family member 6, elongation of long chain fatty acids	*Elovl6*	DOWN	< 0.0001	NM_130450
fatty acid synthase	*Fasn*	DOWN	< 0.0001	NM_007988
stearoyl-coenzyme a desaturase 1	*Scd1*	DOWN	< 0.0001	NM_009127
malic enzyme, supernatant	*Me1*	DOWN	0.00135	NM_008615
sterol regulatory element binding factor 1	*Srebf1*	DOWN	0.00276	NM_011480
atp citrate lyase	*Acly*	DOWN	0.00342	NM_134037
acetyl-coenzyme a carboxylase alpha	*Acaca*	DOWN	0.00888	NM_133360
Fatty acid degradation				
cytochrome P450, family 4, subfamily a, polypeptide 14	*Cyp4a14*	UP	0.00037	NM_007822
carnitine palmitoyltransferase 1a, liver	*Cpt1a*	UP	0.00053	NM_013495
acyl-coa synthetase long-chain family member 4	*Acsl4*	UP	0.00512	NM_001033600, NM_019477, NM_207625
cytochrome p450, family 4, subfamily a, polypeptide 10	*Cyp4a10*	UP	0.00512	NM_010011
acyl-coenzyme a dehydrogenase family, member 9	*Acad9*	DOWN	0.00043	NM_172678
enoyl-coenzyme a, hydratase/ 3-hydroxyacyl coenzyme a dehydrogenase	*Ehhadh*	DOWN	0.00218	NM_023737
acyl-coenzyme A oxidase 2, branched chain	*Acox2*	DOWN	0.00328	NM_053115
Cholesterol synthesis				
3-hydroxy-3-methylglutaryl-coenzyme a reductase	*Hmgcr*	UP	0.00418	NM_008255,
7-dehydrocholesterol reductase	*Dhcr7*	DOWN	< 0.0001	NM_007856
3-hydroxy-3-methylglutaryl-coenzyme a synthase 1	*Hmgcs1*	DOWN	< 0.0001	NM_145942
isopentenyl-diphosphate delta isomerase	*Idi1*	DOWN	< 0.0001	NM_145360, NM_177960
nad(p) dependent steroid dehydrogenase-like	*Nsdhl*	DOWN	< 0.0001	NM_010941
sterol-c5-desaturase (fungal erg3, delta-5-desaturase) homolog (s. cerevisae)	*Sc5d*	DOWN	< 0.0001	NM_172769
sterol-c4-methyl oxidase-like	*Sc4mol*	DOWN	< 0.0001	AK005441
phosphomevalonate kinase	*Pmvk*	DOWN	0.00016	NM_026784
cytochrome p450, family 51	*Cyp51*	DOWN	0.00071	NM_020010
Cholesterol catabolism (bile acid biosynthesis)				
hydroxy-delta-5-steroid dehydrogenase, 3 beta- and steroid delta-isomerase 7	*Hsd3b7*	DOWN	< 0.0001	NM_001040684, NM_133943
lysosomal acid lipase 1	*Lipa*	DOWN	0.00021	AI596237
retinol dehydrogenase 11	*Rdh11*	DOWN	0.00058	NM_021557
sterol o-acyltransferase 2	*Soat2*	DOWN	0.00325	NM_146064
hydroxysteroid (17-beta) dehydrogenase 12	*Hsd17b12*	DOWN	0.00348	NM_019657
cytochrome p450, family 7, subfamily b, polypeptide 1	*Cyp7b1*	DOWN	0.00153	NM_007825

[a]False discoverry rate (FDR) between the control group and the tomato beverage group. In this experiment, genes at FDR < 0.05 were defined as showing biologically significant changes in expression levels. [b]GenBank ID.

Microarray analysis is widely used as a tool to study genes potentially involved in metabolic pathways and homeostatic control. Here, we examined the effects of continuous ingestion of TB on comprehensive gene expression in normal mice, and the DNA microarray analysis revealed down-regulation of sterol regulatory element-binding proteins (SREBPs) and up-regulation of peroxisome proliferator-activated receptors (PPARs) by tomato consumption. In this study, we found that *Srebf1* and most SREBP-1c-responsive genes were consistently down-regulated by TB beverage ingestion (**Tables 4 and 5**). These changes in gene expression strongly suggest the suppressed expression of SREBP-1c. We also found a tendency for up-regulation of the PPARα gene (FDR = 0.01444) and significant up-regulation of the *Cpt1a* gene were observed, indicating that the TB ingestion caused to activated PPARα in the liver of mice. Previous reports have indicated that the overexpression of SREBP-1c in the liver of transgenic mice resulted in the development of a TG-enriched fatty liver[4], and that the absence of SREBP-1 significantly reduced the hepatic expression of lipogenic genes and prevented the development of fatty liver in Leptin-deficient mice[5]. In addition, reductions of fatty acid levels were reported to be affected by the activation of PPARα, which reduces hepatic *de novo* fatty acid synthesis[6,7]. Therefore, it is possible that the decreases in body weight or liver weight loss in the TB group could be attributed to the down-regulation of SREPB-1c and the up-regulation of PPARα.

The main objective of this study is to statistically examine the comprehensive changes in hepatic gene expression caused by the ingestion of TB, to determine the significance of consuming tomato on health. Therefore, we did not explore the identification of any particular active compounds contained in this vegetable that affected gene expression. It was reported that tomato contains valuable components such as lycopene[8], and numerous nutrients and phytochemicals[9]. So, the changes in gene expression observed in this study may be due to additive or interactive effects between each nutrient and/or phytochemical. Meanwhile, some papers have reported that dietary lycopene, the main carotenoid in tomato, affects the expression of genes related to lipid metabolism[10,11]. Therefore, it seems likely that this carotenoid is the principal components that modify gene expression. Further studies on the administration of purified lycopene and the accumulation of lycopene in tissues would clarify the active compounds present in tomatoes.

Although many reports have revealed the benefits of high vegetable diets on health, the genetic pathways through which vegetables exert their effects are still mostly unknown. The modulation of gene expression by dietary tomato has only been investigated in pathological animal models and with a limited number of genes. To our knowledge, the present study is the first to define the effects of tomato on the expression of a large number of genes corresponding to biomarkers in normal mice with microarray technology. The results in this study also demonstrate the importance of consuming tomatoes daily to maintain healthy body conditions and/or reduce the risk of chronic diseases.

Acknowledgement

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Stress Regulation by Inhalation of (*R*)-(–)-Linalool as Seen from Gene Expression Analysis

Akio Nakamura*

1. Introduction

Odorants are low-molecular-weight compounds that offer important information about environments and exogenous substances including foods. It has long been empirically known from ancient times that some odorants bring about psycho-physiological effects, such as sedative, stimulative, anti-stress, and anti-convulsant effects.

Linalool (3,7-dimethyl-1,6-octadien- 3-ol), a monoterpene compound with a floral scent, has been identified in numerous foods and flowers. Its characteristic odor is important not only in formulation of a variety of fruits-flavors and fragrances, but also in eliciting certain kinds of psycho-physiological effects to which a relatively large numbers of studies have been directed[1-6]. It has been suggested that the effects are elicited by its actions on the central nervous systems[3]. Two optical isomers of linalool with (R)- and (S)-configurations act differently on psycho-physiological parameters[4]. (R)-(–)- linalool has been reported to elicit a significant decrease in heart rate under stressed conditions because of having a sedative effect[5].

In recent years, there has been more interest in the psycho-physiological effects elicited by odorants, because they can be reasonably expected to contribute to health maintenance and promotion. However, research for assessing these effects of inhaled odorants in vivo is still quite limited. Therefore, we tried to objectively quantify the effects the inhaled odorant has in vivo by multidisciplinary profiling of blood cells and gene expression. For this purpose, we focused on differences in rats exposed to a two-hour restraint, which was defined as a combination of physical and psychological stressors, with or without exposure to the odorant.

2. Effects of (R)-(–)-linalool inhalation on blood cells and gene expression profiles

Male Wistar rats were divided into four groups (control, stressed, stressed + odorant-inhaled, and odorant-inhaled groups). Rats in the stressed group were placed in a restraining plastic tube for 2 h. Twenty microliters of (*R*)-(–)-linalool (92% ee) were evaporated and allowed to spread throughout a 40-L box containing restrained or non-restrained rats. To verify the effect of the odor inhalation under the stressed condition, we compared the data from the rats among the three treatment groups (control, stressed, and stressed + odorant-inhaled). In neutrophils and lymphocytes, significant changes caused by the restraint were repressed by their exposure to the odorant[7]. This indicates that inhalation attenuates stress-induced changes.

To address the question about whether it would be possible to detect the effect of (*R*)-(–)-linalool on the differential patterns of mRNA expression in whole blood, we compared holistic changes in the mRNA expression among the three treatment groups. Purified total RNA from the whole blood was used to synthesize cDNA and then biotinylated cRNA was transcribed, fragmented, and hybridized to an Affymetrix Rat Genome 230 2.0 GeneChip. One-way ANOVA identified 1695 probe sets in which the mRNA levels were found to be

* Ph.D., Technical Research Institute R&D Center, T. Hasegawa Co., Ltd.

statistically different among the three groups. Heat mapping and hierarchical clustering were then performed based on the data of these 1695 probe sets. The results indicate that the inhaled (*R*)-(–)-linalool has an influence on the gene expression profiles in the restrained rats, although its influence was weaker than that induced by the restraint. Tukey's test which was applied as a post hoc test after one-way ANOVA showed that the applied restraint significantly altered the expression levels of 696 genes, 115 among which were significantly altered by the inhalation. To further assess and clarify the influence of inhaled (*R*)-(–)-linalool on the stress responses induced by the restraint, we examined the expression patterns of the 115 genes. As a result, we noted that (*R*)-(–)-linalool inhalation during the restraint significantly repressed the restraint-induced changes in the expression levels of 109 genes (Figure 1), while it enhanced those of the remaining 6 genes[7].

Figure 1 Illstration of the experimental setup and variation of the 109 gene expression profiles for which the stress-induced changes were repressed by (R)-(–)-linalool in their whole blood. Each line plot shows two-hour-restraint and/or (R)-(–)-linalool-induced changes. The 109 gene expression values passed the filtering criteria of both $p < 0.05$ by Tukey's post hoc test.

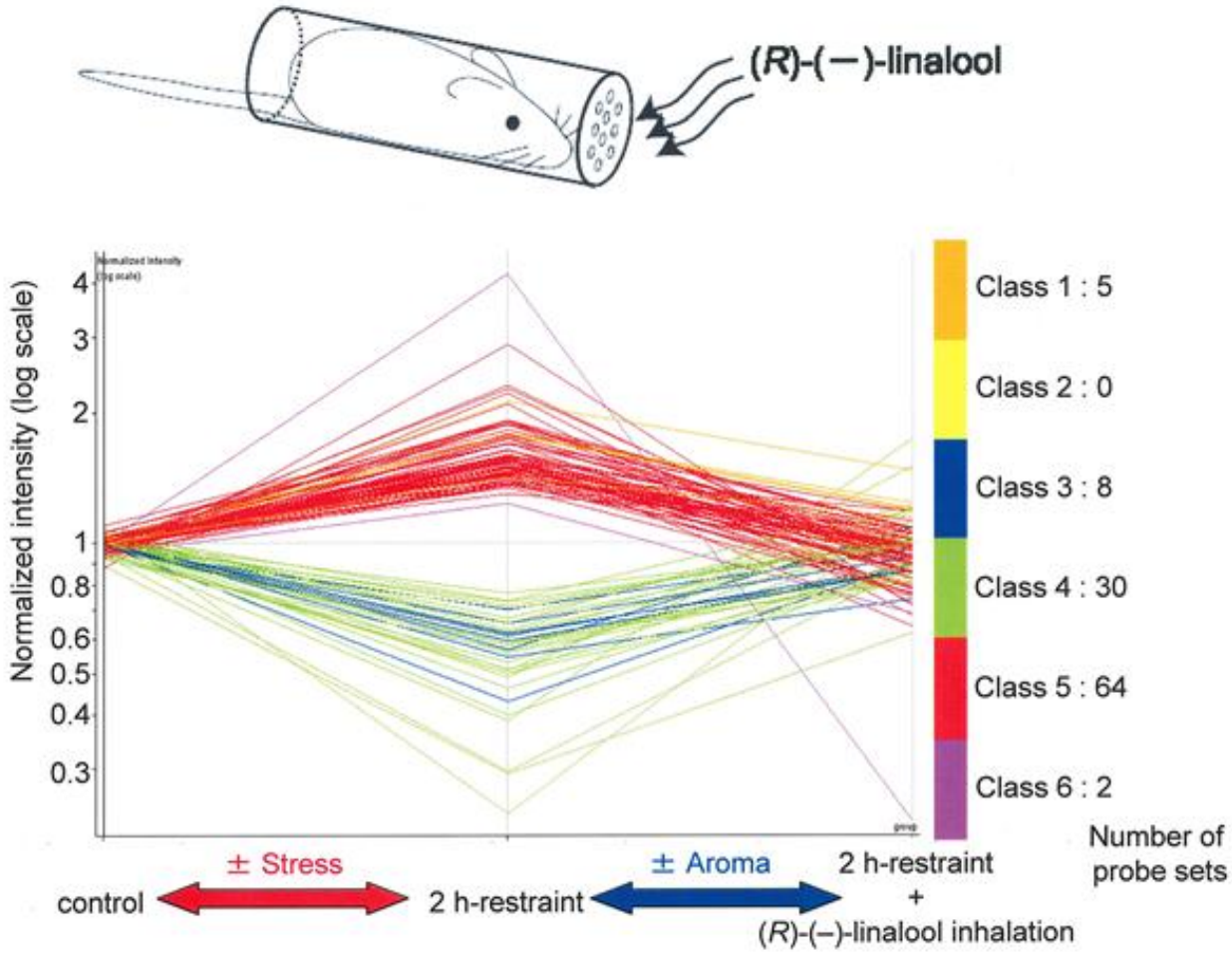

Figure 2 Significantly enriched GO terms found in 594 upregulated genes following (R)-(–)-linalool inhalation under stressed conditions ($p < 0.05$). Counts represent the number of probe sets annotated to each gene ontology (GO) term. FDR-corrected p-values and counts of the categories appearing in the deepest hierarchy are shadowed.

GO-ID	GO term	Counts	FDR-corrected *p*-value
0065007	biological regulation	137	8.42E-03
0050789	regulation of biological process	126	8.85E-03
0019222	regulation of metabolic process	75	1.08E-02
0050794	regulation of cellular process	113	8.16E-03
0031323	regulation of cellular metabolic process	68	2.93E-02
0019219	regulation of nucleobase, nucleoside, nucleotide and nucleic acid metabolic process	63	2.53E-02
0045449	regulation of transcription	62	1.77E-02
0006350	transcription	65	1.96E-02
0010468	regulation of gene expression	67	1.03E-02
0010467	gene expression	85	2.44E-02
0006139	nucleobase, nucleoside, nucleotide and nucleic acid metabolic process	96	9.64E-03
0016070	RNA metabolic process	70	2.44E-02
0009987	cellular process	250	3.27E-02
0030030	cell projection organization and biogenesis	20	2.43E-02
0048858	cell projection morphogenesis	20	2.43E-02
0032990	cell part morphogenesis	20	2.43E-02
0048869	cellular developmental process	65	4.98E-02
0030154	cell differentiation	65	4.98E-02
0031175	neurite development	17	3.14E-02
0048666	neuron development	19	2.49E-02
0030182	neuron differentiation	22	2.44E-02
0048699	generation of neurons	24	2.57E-02
0022008	neurogenesis	25	3.40E-02
0007399	nervous system development	39	3.32E-02

3. Effects of (R)-(–)-linalool inhalation on gene expression profiles in the hypothalamus

(*R*)-(–)-linalool has a partially repressive effect on changes induced by the stress in the gene expression levels to restore them to their normal levels over the course of a 2-h restraint period. However, it remained unclear how the complicated relationships of the gene network mechanisms are induced in the CNS just by odor inhalation for 2 h. Therefore, another study was carried out by gene expression profiling for a sample of hypothalamus as a stress response center[8].

A hierarchical clustering analysis using the normalized values obtained by the RMA algorithm from all 31,099 probe sets revealed that inhaled (*R*)-(–)-linalool influenced the gene expression profiles in hypothalamic tissues. The differentially expressed genes by (*R*)-(–)-linalool inhalation under the restrained stress were classified into functional categories according to gene ontology (GO) (Figure 2). The most specifically overrepresented categories in the upregulated genes with the deepest hierarchy involved neurite development, regulation of transcription, cell projection morphogenesis, and RNA metabolic processes[9]. These GO terms fell into two clusters: neuron differentiation and regulation of gene expression.

Furthermore, the inhalation of (*R*)-(–)-linalool during the 2-h restraint promoted some stress responses. Intriguingly, the upregulated genes included a number of heat shock proteins (HSPs) (*Hspa1L*, *Hspb1*, *Hsph1*, *Dnajb1*), CCAAT/enhancer binding proteins (*Cebpb*, *Cebpd*), a Homer homolog (*Homer1*) and IEG (*Fos*)[9]. It is known that HSPs are important modulators of the apoptotic pathway whose cytoprotective functions are largely ex-

plained by their anti-apoptotic function[10]. Accordingly, these genes play a protective role in helping the cell to cope with lethal conditions and (*R*)-(–)-linalool at least partially enhanced the defense response.

4. Regulation of stress by odorants

The present study is an attempt aiming to quantitatively analyze psycho-physiological effects of odorants. For this purpose, we profiled blood cells and gene expression in the whole blood and hypothalamus of restrained rats which inhaled the vapors of (*R*)-(–)-linalool. This profiling revealed a repressive effect by (*R*)-(–)-linalool on the stress-induced changes in blood cells and in gene expression levels. We have shown that (*R*)-(–)-linalool inhalation returned stress-elevated levels of neutrophils and lymphocytes to near-normal levels. Inhaling linalool also reduced the activity of more than 100 genes that go into overdrive in stressful situations using their whole blood of the odorant-inhaling rats subjected to acute restraint stress. Another study, aiming to explain one of molecular logics of stress relaxation by (*R*)-(–)-linalool inhalation, was carried out by gene expression profiling with a sample of hypothalamus as a stress response center. The following two conclusions were thus obtained: (1) inhalation of this aroma under a restraint stress-added condition up-regulated a number of neuron differentiation-related genes toward activating the processes of neuronal maturation; and (2) the inhalation also up-regulated restraint stress-inducible, heat shock protein-related genes that can be associated with the suppression of stress-caused apoptosis.

Our findings has elucidated a physiological effect of an inhaled pleasant odor, (*R*)-(–)-linalool in this case, by an in-depth analysis of gene expressions, and also could largely contribute as a new method for evaluating in vivo effects caused by odorants to cope with stresses.

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Hepatic gene expression analyses for assessment of a reference iron intake

Asuka Kamei*

1. Iron

Iron is an essential modulator of metabolic and physiological functions through its role as a cofactor for many of proteins[1, 2]. If for any reason iron deficiency occurs, iron stored in organs such as the liver is utilized to compensate for the deficiency. This compensation is particularly important for animals (including humans) during development. Longer periods of iron deficiency result in a smaller iron storage pool, suppressing hemoglobin biosynthesis with occurrence of anemia. Iron deficiency-induced anemia in associated with down-regulation of blood hemoglobin level, with a serious result which is already a public health problem in developed as well as developing countries. Globally, anemia affects 1.62 billion people corresponding to 24.8% of the population (1993-2005, WHO). The highest prevalence occurrence is seen in preschool-age children (47.4%), and the lowest one in adult males (12.7%).

2. Iron-deficiency anemia

Down-regulation of hemoglobin level caused by iron deficiency leads to changed energy metabolism in the peripheral tissue because the efficiency of oxygen transport to this tissue is decreased. However, no global analysis detailing the consequences of iron deficiency in the liver has been conducted yet. Since the liver is a metabolically important organ and also a major iron-storing organ, we performed a comprehensive transcriptome analysis to determine the effects of iron deficiency on hepatic gene expression.

2.1 Animals

Four-week-old rats were fed an iron-deficient diet (approximately 3 ppm iron) *ad libitum* for 16 days. These rats were compared with those pair-fed a control, normal iron level (48 ppm iron). The feeding with the 16-day iron-deficient diet apparently induced anemia (Table 1).

Table 1 Body weight, liver weight, hemoglobin, serum iron and liver iron in rats

Group	n	Body weight	Liver weight	Hemoglobin	Serum iron	Liver iron
		g	g	g/dl	μg/dl	μg/g wet tissue
Pair-fed group	6	194.5 ± 1.2	9.0 ± 0.3	14.4 ± 0.4	247.0 ± 29.0	101.8±5.6
Iron-deficient	7	198.5 ± 5.7	7.9 ± 0.4*	6.1 ± 0.2**	35.0 ± 3.4**	66.0±3.6**

The values represent the mean ± SEM. * $P < 0.05$ and ** $P < 0.01$ for between-diet group differences.

* Project on Health and Anti-aging, Kanagawa Academy of Science and Technology

Table 2 Significantly enriched GO terms (FDR-corrected P -value < 0.01) found in the top 600 upregulated genes in the iron-deficient group

GO_ID	Term	FDR-corrected *P* -value
0002376	immune system process	8.18E-06
0019882	antigen processing and presentation	1.63E-06
0048002	antigen processing and presentation of peptide antigen	1.83E-06
0002474	antigen processing and presentation of peptide antigen via MHC class I	1.73E-06
0006955	immune response	1.05E-04
0050896	response to stimulus	1.62E-04
0006950	response to stress	4.95E-03
0006952	defense response	2.35E-04
0002526	acute inflammatory response	1.32E-03
0006954	inflammatory response	1.78E-04
0009611	response to wounding	9.95E-05
0009605	response to external stimulus	9.98E-05
0008152	metabolic process	
0006629	lipid metabolic process	6.57E-03
0008610	lipid biosynthetic process	9.82E-03
0044255	cellular lipid metabolic process	3.00E-04
0008202	steroid metabolic process	1.01E-07
0006694	steroid biosynthetic process	1.89E-04
0016125	sterol metabolic process	3.07E-07
0016126	sterol biosynthetic process	1.00E-04
0008203	cholesterol metabolic process	1.16E-05
0006066	cellular alcohol metabolic process	1.45E-06
0006082	organic acid metabolic process	7.70E-04
0019752	carboxylic acid metabolic process	6.97E-04
0009987	cellular process	
0007165	signal transduction	
0007249	I-kappaB kinase/NF-kappaB cascade	6.40E-03
0016265	death	
0006915	apoptosis	
0051402	neuron apoptosis	1.09E-03
0065007	biological regulation	
0050793	regulation of developmental process	
0043523	regulation of neuron apoptosis	3.28E-03
0043525	positive regulation of neuron apoptosis	1.92E-03
0048518	positive regulation of biological process	1.81E-03
0045787	positive regulation of progression through cell cycle	7.31E-03
0050794	regulation of cellular process	
0050806	positive regulation of synaptic transmission	7.88E-03
0051239	regulation of multicellular organismal process	

FDR-corrected *P* -values were defined by the modified Fisher's exact test with Benjamini and Hochberg FDR correction.

FDR-corrected *P* -values of the categories appearing deepest in the hierarchy are shadowed.

2.2 DNA microarray analysis

To determine the effects of iron deficient anemia on hepatic gene expression, comprehensive transcriptome analysis was performed by DNA microarray (Affymetrix GeneChip® Rat Genome 230 2.0 Array). Hierarchical clustering analysis revealed that each experimental group formed a large cluster of its own (Figure 1). We identified 600 up-regulated and 500 down- regulated probe sets that characterized the iron-deficient diet group. In the up-regulated probe sets, genes involved in cholesterol, amino acid, and glucose metabolism were significantly enriched (Table 2), while those related to lipid metabolism were significantly enriched in the down-regulated probe sets (Table 3). Gene lists are summarized in Tables 4 and 5. Metabolites from these metabolic processes in the liver and serum were measured (Table 6) and possible hepatic metabolic changes due to dietary iron deficient anemia are summarized in Figure 2. We also found that genes for caspases 3 and 12, which mediate endoplasmic reticulum (ER)-specific apoptosis, were up-regulated in the iron-deficient group (Figure 3). ER stress response is induced by accumulation of unfolded proteins. Our data showed that gene expression levels of Hspa1a and Hspa1b, members of the chaperone protein family, decreased significantly. This triggers the accumulation of unfolded proteins, resulting in ER stress.

Our study is the first to demonstrate that iron deficient anemia simultaneously influences a wide range of nutrient metabolism and even apoptosis as a consequence of ER stress[3].

3. Non-anemic iron deficiency

In Japan, about 10% of women suffer from iron-deficient anemia, and 20-40% from non-anemic iron deficiency[4] which does not appear to cause any serious problems because no appreciable down-regulation of hemoglobin occurs. However, iron is essential for biochemical activation of cytochrome-related enzymes and its deficiency may cause serious physiological problems. To know this status at molecular level, we performed global transcriptomics by DNA microarray analysis, with successful results.

4. Iron overload

Intestinal absorption of iron is controlled strictly. Ingestion of an excess of iron causes inhibition of its intestinal absorption. On the other hand, excessive accumulation of iron in organs can cause oxidative damage. To look at influences of excessive iron intake on the body at molecular level, we performed global transcriptomics by DNA microarray analysis to provide some food safety information.

Table 3 Significantly enriched GO terms (FDR-corrected P -value < 0.01) in the top 500down-regulated genes in the iron-deficient group

GO_ID	Term	FDR-corrected *P* -value
0006082	organic acid metabolic process	2.59E-09
0019752	carboxylic acid metabolic process	4.20E-09
0032787	monocarboxylic acid metabolic process	1.83E-06
0006631	fatty acid metabolic process	1.13E-04
0044255	cellular lipid metabolic process	2.80E-04
0006629	lipid metabolic process	2.47E-04
0008610	lipid biosynthetic process	6.68E-03

See Table 2 for FDR-corrected *P* -value definition.
See Table 2 for an explanation of shadowed *P* -value representation.

Figure 1 Hierarchical clustering dendrograms from the DFW-quantified DNA microarray data. Pair-fed, pair-fed group; Iron-def, iron-deficient group. Numbers represent independent samples. The vertical scale represents between-cluster distances.

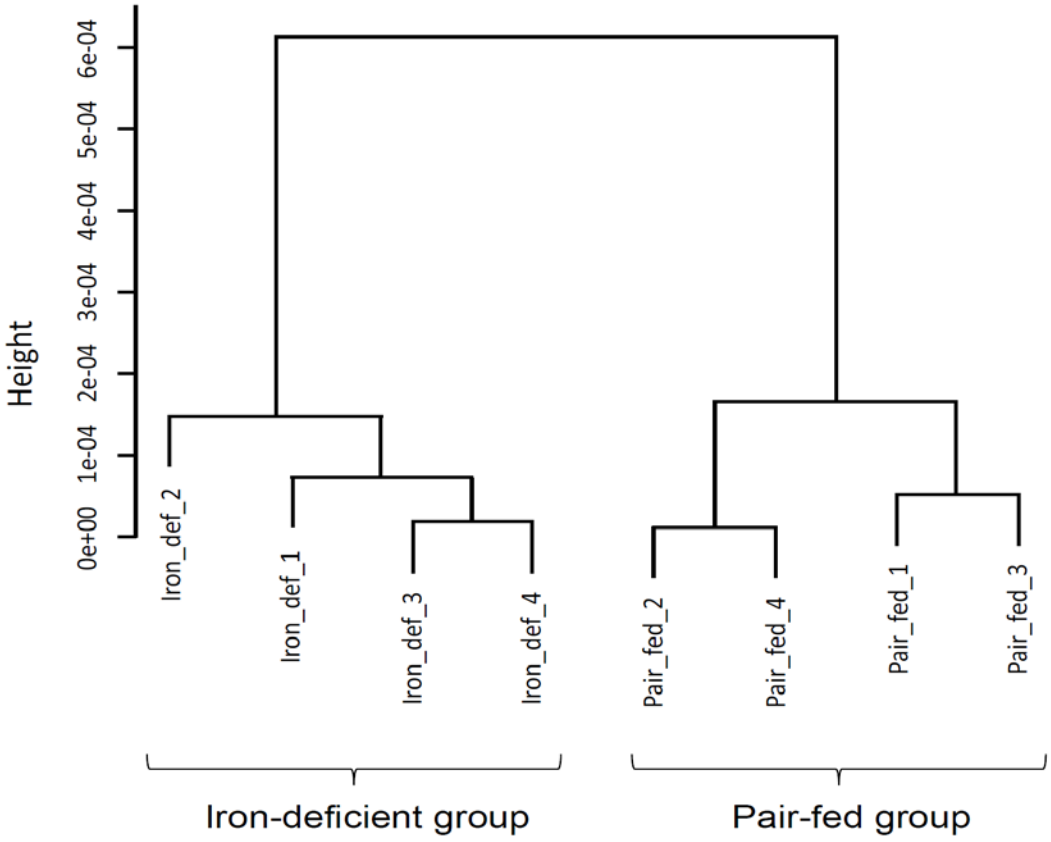


Figure 2 Possible hepatic metabolic changes due to dietary iron deficient anemia. Orange arrows, up-regulated gene expression; blue dashed arrows, downregulated gene expression. Orange squares, increased level in serum or liver; blue squares, decreased level in serum or liver.

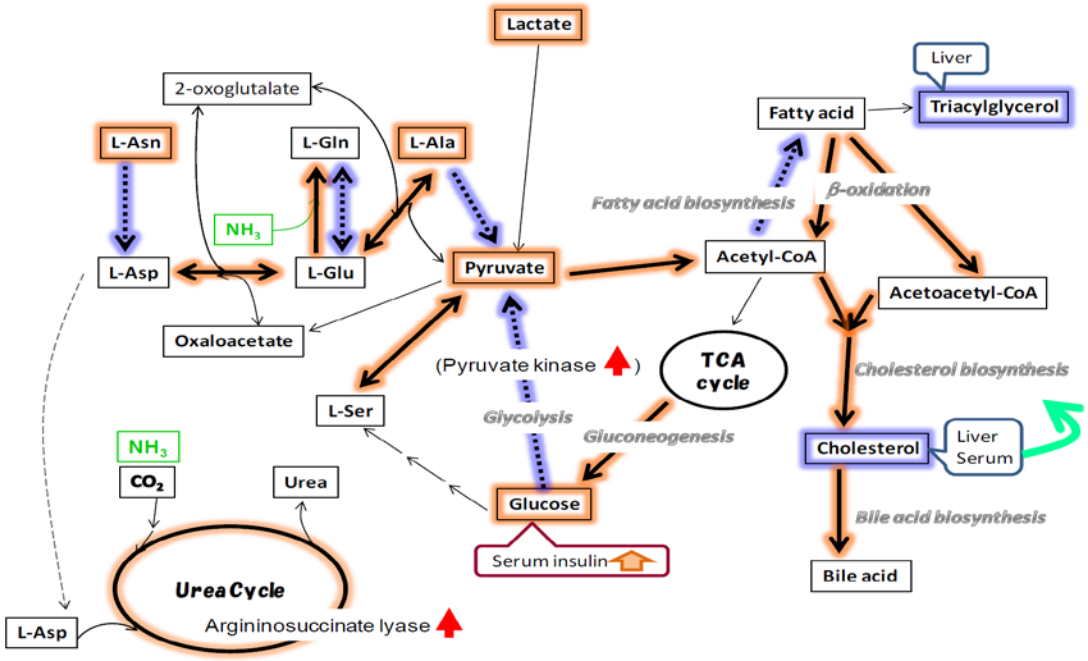


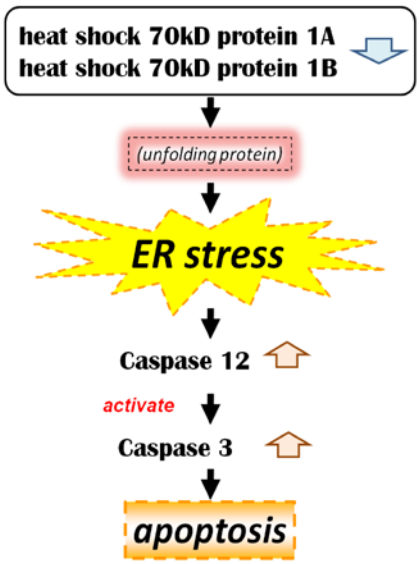
Table 4 List of genes with increased expression in livers of rats fed iron-deficient diet

Gene Title	Public ID
Cholesterol metabolic process	
Sterol biosynthetic process	
3-hydroxy-3-methylglutaryl-coenzyme A synthase 1	NM_017268
3-hydroxy-3-methylglutaryl-coenzyme A reductase	BM390399
isopentenyl-diphosphate delta isomerase	NM_053539
farnesyl diphosphate synthetase	NM_031840
squalene epoxidase	NM_017136
cytochrome P450, subfamily 51	NM_012941
24-dehydrocholesterol reductase	BF417479
sterol-C4-methyl oxidase-like	NM_080886
NAD(P) dependent steroid dehydrogenase-like	BF407232
cytochrome P450, family 7, subfamily a, polypeptide 1	NM_012942
insulin induced gene 1	NM_022392
insulin induced gene 2	AA851803
Carboxylic acid metabolic process	
Amino acid metabolic process	
glutamate-ammonia ligase (glutamine synthetase)	NM_017073
serine dehydratase	NM_053962
glutamic pyruvic transaminase 1, soluble	NM_031039
glutamate oxaloacetate transaminase 1, soluble	D00252
argininosuccinate lyase	NM_021577
Gluconeogenesis	
glucose-6-phosphatase, catalytic	NM_013098
phosphoenolpyruvate carboxykinase 1, cytosolic	BI277460
Acute inflammatory response	
complement component 1, s subcomponent	D88250
complement component 1, r subcomponent	BI292425
complement component 4, gene 2	BI285347
complement component 6	AI045191
Apoptosis	
caspase 3, apoptosis related cysteine protease	U84410
caspase 12	NM_130422
Neuron apoptosis	
BCL2/adenovirus E1B 19 kDa-interacting protein 3	NM_053420

Table 5 List of genes with decreased expression in livers of rats fed iron-deficient diet

Gene Title	Public ID
Fatty acid metabolic process	
Lipid biosynthetic process	
fatty acid synthase	NM_017332
stearoyl-coenzyme A desaturase 1	J02585
fatty acid desaturase 1	NM_053445
fatty acid desaturase 2	NM_031344
sterol regulatory element binding factor 1	AF286470

Figure 3 Possible induction of apoptosis as a consequence of endoplasmic reticulum (ER) stress due to dietary iron deficient anemia.
Orange arrows, up-regulated gene expression; blue dashed arrows, down-regulated gene expression.



5. Conclusion

The importance of accumulating the data on various degrees of iron status in the body from a nutrigenomic point of view is emphasized. Our studies on the influence of iron at molecular level would be applicable to assessing the requirements of other nutrients in general.

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Chinese strategy on anti-aging research trends

Zhengwei Fu*

The dramatic fertility decline and improved longevity over the past two decades are causing China's population to aging at one of the fastest rates ever recorded according to the census conducted last year. This change is accompanied by an increase in the prevalence of chronic disease and disability in the population. The Chinese government responses to population aging are strategies such as perfection of pension system, prevention for chronic disease, and anti-aging studies. Here we briefly review aging theories and related genes, aging animal models, anti-aging research and trends in China. Western medical theories of aging like free radical theory, mitochondrial DNA damage are widely accepted by Chinese researchers. Previous studies have shown that aging related genes like Klotho, Sirt1, and miRNA are playing crucial roles in chronic disease associated with senescence. This kind of gene mutant or knockout could induce resemble observations in the normal senescence process, which is often used to build aging animal models. Moreover, scientists in China first reported an effective aging model induced by injection of D-galactose (D-gal) in 1980s, which is still widely used in China's anti-aging research. And this D-gal induced aging model is recently improved by a combination with jet-lag exposure. Based on these aging theories and animal models, different therapies like food restriction therapy, antioxidant treatment, hormone therapy became modern anti-aging effective methods in China. On the other hand, in Traditional Chinese Medicine (TCM), kidney-deficiency was thought to be the main cause of senescence. Numerous studies were carried out to have a comprehensive understanding of the aging mechanism and search for anti-aging products through TCM theories. Single Chinese herbs like Goji berries, Ginseng showed good effect in curing chronic disease and preventing senescence. However, in order to accentuate efficacy as well as to reduce side effects, Chinese herbal therapies are generally based on herbal formula not merely on a single herb. Furthermore, Chinese people also chase for the anti-aging functional food. Chinese medicinal cuisine is unique in the world, which produces functional health food by combining herbal ingredients with traditional culinary materials. Anti-aging strategies have evolved in China, while the aging mechanism, anti-aging products still need to be comprehensively, systematically, and carefully researched.

China's census conducted late last year showed its population grew to 1.34 billion by 2010, with a sharp rise in those over 60, which is now account for 13.3% of the population, up nearly 3% since 2000. The percentage is expected to reach 16.7% in the next five years, and about 30% by 2050. Because chronic health problems become more common in old age, China's population aging has led to the increase in the country's prevalence of chronic disease and disability. Moreover, improved living standards in China have exacerbated the epidemic of chronic disease by increasing exposure to major risk factors such as smoking, high-fat and high-calorie diets, and more leisure time without physical activity. While the trend of population aging is inevitable and can even be accelerated by further declines in mortality and

* Professor, PhD, College of Biological and Environmental Engineering, Zhejiang University of Technology, Hangzhou, Zhejiang, 310032, China

fertility, stemming the epidemic of chronic disease is one promising way to reduce the overall impact of aging on China's social and economic development.

Anti-aging medicine, also known as preventative medicine, makes it possible to slow, stop and substantially reverse the physical deterioration, which are commonly considered "natural" aging. Such progress allows not just for an extended existence, but for the enjoyment of optimum wellness and quality of life. China has a great demand for this type of integrative health care, because the country's rapid increase of health problems is related to a longer life expectancy - 81 years now in Shanghai - as well as the prevalence of chronic diseases like respiratory illnesses, cancer, and diabetes. In a word, Chinese anti-aging strategy is of prime importance not only for Chinese people, but also for anti-aging research all over the world.

1. Mechanisms of Aging

1.1 Medical Theories of Aging

Denham Harman first proposed the free radical theory of aging in the 1950s[1], and in the 1970s extended the idea to implicate mitochondrial production of reactive oxygen species (ROS) [2]. After that more and more theories were proposed to clarify the mechanism of aging, such as mitochondrial DNA damage, cross linkage theory, biological membrane damage, genetic program theory. Among all these, oxidative stress caused by free radical and mitochondrial damage is still postulated to be a major causal factor of senescence, which is widely accepted and used in China's anti-aging research.

Besides the western medical theories, China also has its own traditional Chinese medicine (TCM) theories of aging for thousands of years, which is another research emphasis to clarify the aging mechanism and search for anti-aging products. In TCM, Kidney-Deficiency is thought to be a main cause of senescence since "Yellow Emperor's Internal Classic", dating back to about 200 B.C., which suggests that it is extremely important to tonify the kidney. According to TCM theories, kidney stores essence, rules water metabolism, grasps Qi, manifests in the hair, and opens into the ears and genital organs. Kidney deterioration is regarded as the root of aging, its associated signs include diminished hormone levels, overall decrease in energy and vigor, loss of bone strength and density, shrinkage in the sexual organs, changes in the menstrual cycle, urinary problems, loose teeth, limb soreness, impaired hearing, gray and thinning hair. Combined with Yin Yang theory, numerous studies were performed to elucidate the aging theories from different aspects, such as kidney Qi deficiency, kidney Yang deficiency, Qi deficiency in the kidney and spleen, Yang deficiency in the kidney and spleen, kidney Yin deficiency, Yin deficiency in kidney and lung, Yin deficiency in kidney and liver. Based on these different medical theories, Chinese anti-aging researches are making great progress both in having a better understanding of aging mechanism and searching for effective anti-aging products.

1.2 *Klotho* and Aging

Deficient in *Klotho* gene expression in mice results in a syndrome that resembles human aging and induces a short lifespan, infertility, arteriosclerosis, skin atrophy, osteoporosis and emphysema. Two notable changes in Klotho-deficient mice are arteriosclerosis and endothelial dysfunction, which are two fundamental etiological factors of essential hypertension. Scientists of Third Military Medical University of China find that the G-395A polymorphism of the human *Klotho* gene is associated with essential hypertension and might be a potential regulatory site [3].

For a better understanding the function of *Klotho* gene and aging mechanisms, a cell-based assay for high-throughput drug screening is established by Chongqing University to identify compounds that could regulate *Klotho* promoter activity. With this assay it may be possible to separate and identify the active compound responsible for the observed effect from other natural products extract [4].

Senescence-related DNA damage seems to be a hallmark of many cancers. The relationship between senescence and tumorigenesis is close but highly complicated. Recently, growing evi-

dences demonstrated that impaired *Klotho* function not only induces the aging process but also contributes to tumorigenesis [5]. *Klotho* is found to be downregulated in breast cancer tissues compared with normal samples, and over-expression of *Klotho* suppresses cell proliferation in breast, lung, and cervical cancer cells lines [6]. Researchers of Zhejiang University demonstrate the correlation of *Klotho* and colorectal cancer, which suggests that *Klotho* is inactivated through promoter hypermethylation and potentially functioned as a tumor suppressor gene in colorectal cancer [7]. Further studies on the *Klotho* family are expected to provide new insights into endocrine regulation of various metabolic and aging processes.

1.3 SIRT1 and Aging

SIRT1 is an important determinant of longevity that plays a role in life-span regulation in diverse species. This gene has been extensively investigated and shown to delay senescence. However, whether SIRT1 has a function to influence cell viability and senescence under non-stressed conditions in human diploid fibroblasts is far from known. Tong and his colleagues show that enforced SIRT1 expression promotes cell proliferation and antagonized cellular senescence with the characteristic features of delayed Senescence-Associated β-galactosidase (SA-β-gal) staining, reduced Senescence-Associated Heterochromatic Foci (SAHF) formation and G1 phase arrest, increased cell growth rate and extended cellular lifespan in human fibroblasts[8]. Further research implies the delay of senescence by SIRT1 is associated with downregulation of $p16^{INK4A}$/Rb pathway and the activation of ERK/S6K1 signaling. The decline of SIRT1-dependent ERK/S6K1 signaling in senescent cell may contribute to cell progression loss and cellular senescence at late passage of human diploid fibroblasts.

In addition, the regulation of SIRT1 during aging is also less understood. Tong et al show that PPARγ inhibits SIRT1 expression at the transcriptional level, in part by deacetylation. Moreover, both PPARγ and SIRT1 can bind the SIRT1 promoter. PPARγ directly interacts with SIRT1 and inhibits SIRT1 activity, forming a negative feedback and self-regulation loop [8]. This model may offer an opportunity to observe gene-environment interactions associated with cellular senescence in health and disease.

Zu and his colleagues find that Liver Kinase B1 (LKB1) is a potential intracellular target of SIRT1 [10]. Moreover, the protective activities of SIRT1 may be achieved at least in part by fine tuning the acetylation/deacetylation status and stabilities of LKB1 protein, antagonizing LKB1-mediated AMPK signaling pathways. Further investigation about the specific mechanism of SIRT1 is required, which may be used as a new anti-aging drug target.

1.4 miRNA and Aging

Although the relationship between miRNA and ageing is not fully understood, studies have provided evidence showing that miRNAs participate in regulating cell cycle progression, proliferation, stemness gene expression, and stress-induced responses. Since some miRNAs are tumor suppressors or proto-oncogenes, the possibility exists that certain miRNAs may be critical determinants of aging.

Chinese Academy of Sciences survey the gene and miRNA expressions in representative epididymides encompassing the whole human lifespan, and find out that the newborn human epididymis expressed the fewest mRNAs but the largest number of miRNAs, whereas the adult and aged epididymides expressed the most mRNAs but the fewest miRNAs, a negative correlation between mRNAs and miRNA during aging(11). It also provided novel insights into the temporal and androgen-dependent gene/miRNA expression involved in the development and aging processes in the human epididymis[11].

Bai and his colleagues suggest that superoxide dismutase 2 (SOD2) and thioredoxin reductase 2 (Txnrd2), located in the mitochondria, are potential targets of miR-335 and miR-34a respectively by bioinformatics analysis [12]. The results also indicated that miRNAs may contribute to renal aging by inhibiting intracellular pathways such as those involving the mitochondrial antioxidative enzymes SOD2 and

Txnrd2.

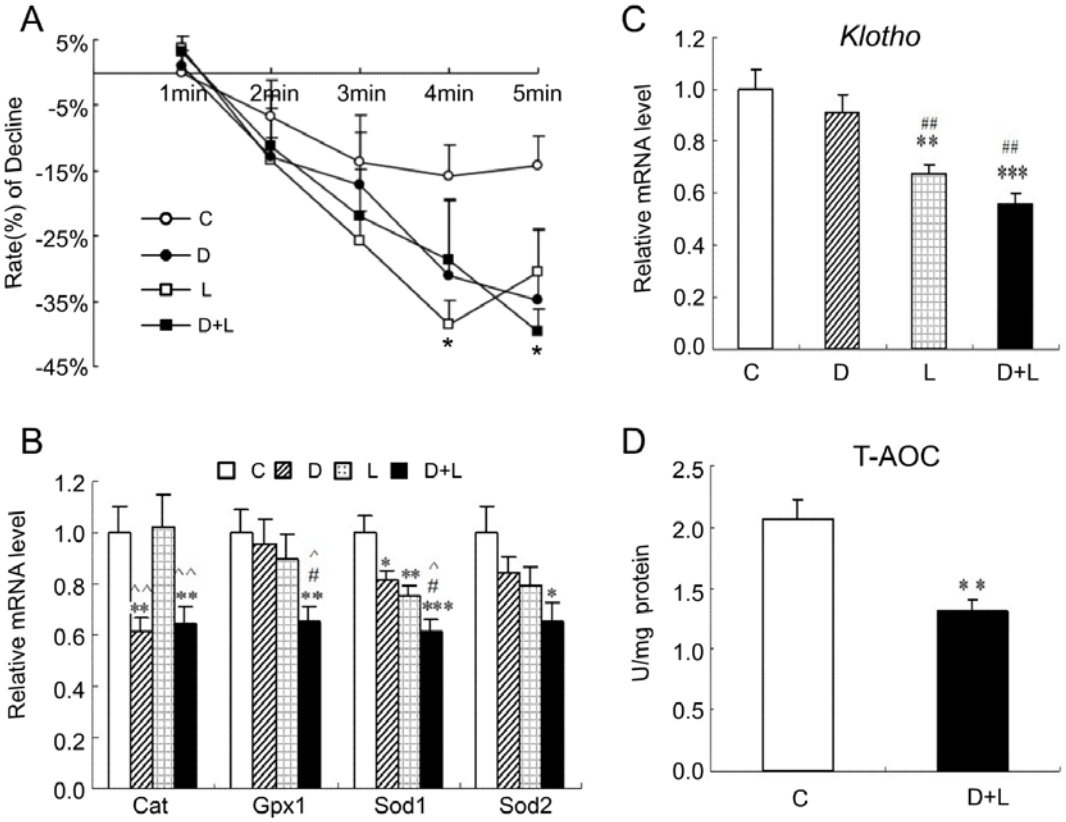
Xiamen University's research showed the role of miR-17-92 in suppressing oncogene-induced senescence, and the anti- senescence activity of miR-17-92 is mediated by the miR-17/20a components [13]. Furthermore, the miR-17-92 cluster and its miR-17/20a components conferred resistance to oncogene-induced senescence at least partly by directly targeting $p21^{WAF1}$, a key effector of senescence induction. Molecular studies of ageing and miRNAs would provide a more comprehensive understanding of the mechanisms of ageing and, subsequently, help to ameliorate this universal process compromising our quality of life.

2. Experimental Model of Aging

Laboratory mice provide a useful model system for the study of the mechanisms of senescence of the organism. Many inbred mouse strains with different senescence profile have been established and utilized for genetic study of lifespan, such as senescence accelerated mouse (SAM) strains, a murine model of accelerated senescence, consist of series of SAMP (prone) and SAMR (resistant) lines, which are widely used in Japan, USA, and other countries. Others like Klotho mutant mice, Mn-SOD conditional knockout mice, senescence marker protein-30 (SMP30) knockout mice are also utilized for better understanding of mechanisms of senescence or age-associated disorders.

It is first reported in China in 1985 that injection of a low dose of D-galactose (D-gal) into mice can induce symptoms which resemble accelerated aging, such as neurological impairment, decreased activity of anti-oxidant enzymes, and poor immune responses[14, 15]. This model has been widely used for aging research and drug testing thereafter. In the past few decades, hundreds of publications had appeared in the Chinese medical literatures using this model.

Figure 1 Effect of jet lag, D-gal or both exposures on (A) the locomotive muscular function by swimming test, (B) antioxidant genes expression in liver, (C) Klotho gene expression in kidney, (D) T-AOC of mitochondria in hepatocytes. Data represents as mean ± SE. * P<0.05, ** P<0.01, *** P<0.001 versus C; # P<0.05, ## P<0.01 versus D; ^ P<0.05, ^^ P<0.01 versus L.



It has been found in our studies that combined chronic D-gal treatment with jet-lag exposure is more effective in establishing the oxidative damage in the accelerated aging model[16]. Briefly, 12-weeks old male ICR mice were divided into 4 groups: control group (C), D-gal (500 mg/kg bw) treated group (D), jet lag exposed group (L), which was carried out by a 12 h-reversal of the light cycle once every 3 days, and both D-gal and jet lag exposure group (D+L). When D-gal treatment was accompanied with a simultaneous jet lag exposure, it decreased mRNA level of antioxidative genes in liver, reduced *Klotho* gene expression in kidney, and lowered total antioxidant capabilities (T-AOC) of mitochondria in hepatocytes were found (Fig. 1), although the jet lag alone didn't have any effect on locomotive muscular function in accelerating the aging process. These results suggested that the jet lag could accelerate the D-gal-induced aging process.

In addition to the D-gal induced aging model, other animal models like thymectomized rat model, which will cause immunocompromised or immunodeficiency that will accelerate the aging process, and O_3 inhalation or γ-ray irradiation which will induce oxidative damage based on free radical theory, are also used for establishment of aging model in China.

3. Advance on Chinese Anti-aging Research

3.1 Gene Polymorphism and Natural Longevity

In China, different age groups of old people may be influenced by different factors. For people aged 65 and above, their distribution may be more influenced by the economical factor, decreasing from east to west. However, for people aged 100 and above, they may be more influenced by the environmental factors. The number of centenarians per 100,000 is opposite to the percentage of people above 65, decreasing from west to east. On the other hand, the centenarian who represents the extremity of longevity, was the optimal population of researching the human longevity. The quantity and relative ratio of centenarian in Xinjiang Hotan is over the average level of whole country, which is classified as one of four longevity regions in the world. Furthermore, because of environment, living characteristics, customs and habits, the Uygur who lived in this region became genetic isolation population and belonged to the national longevity, became valuable resource of genetic study about longevity.

Researchers in Xinjiang Medical University have investigated the association between gene polymorphism and longevity. They find that AA genotype of 5178A/C and GG genotype of 10398G/A polymorphism within mtDNA, AA genotype of promoter-2437G/A polymorphism within HSP gene, AA genotype of promoter-866G/A polymorphism within UCP2 gene, are the advantageous factor of longevity, resulted from long-term genetic isolation in Uygur in Xinjiang Hotan . Others like TERT gene rs2736100 and rs2075786 polymorphisms, VEGF gene rs2146323 polymorphism might be associated with longevity through impacts on vascular function.

3.2 Anti-aging Medicine

In recent years, the development of life science and biotechnology have offered strong support for research of the anti-ageing medicine in China. Food restriction therapy, antioxidant treatment and hormone therapy have already become modern anti-aging effective methods. Regular intake of microelements related to the longevity like ferrum, selenium, magnesium, manganese, copper, zinc, are essential for many organism function, as well as antioxidative effect. Most of these microelement supplement is using compound preparation in Chinese market, such as compound selenium tablets, sodium selenite tablets, geriatric capsules. Others like multivitamins compound, vitamins and microelement compound are also commonly found both domestic and overseas to meet the daily supplement of the elder. Among all the hormone therapies, melatonin is a prime candidate for slowing the aging process and targeting its underlying pathology. Nao-Bai-Jin (implying it is platinum for brain) is a functional product in China , the main component of which is melatonin. It has been claimed to have a great effect

in improving sleeping disorder and anti-aging. It is highly praised and appreciated by the consuming public all over the country, which becomes a household word.

Moreover, scientists in China have achieved great progress in using single Chinese herb to treat different kinds of diseases in the last few decades. One of the most famous and popular is Goji berries, which is also sold as a health food product in western countries. It is praised in advertisements and media for well-being and as an anti-aging remedy since the beginning of this century. Investigations of the fruit have focused on proteoglycans, known as "Lycium barbarum polysaccharides", which show antioxidative properties and some interesting pharmacological activities in the context of age related diseases such as atherosclerosis and diabetes[17]. Another well known single herb is Ginseng, which contains many bioactive constituents, including various ginsenosides that are believed to have antioxidant, anti-inflammatory, anticancer, immunostimulatory, and anti-aging activities[18]. Panax ginseng is one of the most commonly used Chinese medicines in China, Asia and Western countries. Results of clinical research studies demonstrated that Panax ginseng may improve psychologic function, immune function, and conditions associated with diabetes[19]. More commonly known as Reishi, ganoderma is a hard, bitter mushroom used to promote health and longevity. It can relieve fatigue, reduce cholesterol level, lower blood pressure, tame inflammation and enhance immune function. Evidences have shown four novel ergosterol derivatives in the ganoderma, ganodermasides A, B, C and D, which can extend the replicative life span of yeast by regulating *UTH1* expression[20, 21]. Other single Chinese herbs like schisandra, Chinese angelica, tuber of multiflower knotweed, codonopsis pilosula are also believed to improve SOD activity and remove free radicals in brain and liver, which slows down the aging process.

On the other hand, herbal therapies are generally formula based, herbs are seldom singlely used in TCM. TCM holds that every medicinal substance has its strengths and its shortcomings, and each ingredient in the formula should be carefully balanced in quality and quantity, in order to accentuate its efficacy while reducing side effects, thus is more widely used for anti-aging. Six-ingredient Rehmannia Pill which contains processed rhemannia rhizome, cornus fruit, Chinese yam, oriental water-plantain rhizome, poria, peony root bark, is used to resist aging related disease like nervous debility, diabetes mellitus, hyperthyroidism, Addison's disease, chronic nephritis, chronic urinary tract infection, hypertension. Processed rhemannia rhizome and Chinese yam in the pill are also believed to have the ability to remove the free radicals. Four-gentlemen Decoction which contains ginseng, largehead atractylodes rhizome, poria, roasted liquorice root, is used for paleness, feeble voice, limb weakness, reduced appetite, weak pulse, which are all related to senium.

However, because the herbal compound is produced by the large amount of ingredients, proportion of ingredients and complicated production processes, series of different process techniques are developed to make the compound keep its efficacy. Qingchunbao(青春宝) Anti-aging Tablet, just as its name implies, is a drug with distinctive effect of the long maintenance of youth and the postponement of aging. This drug is formerly an imperial nostrum in Ming Dynasty of China, and its formular contains a series of ingredients including radix ginseng rubra, radix rehmanniae, radix asparagi, radix ophiopogonis, cortex lycii, poria. It can supply the vital energy, nourish the vital essence, and relieve mental stress. It is commonly used by the aged and middle-aged people suffering from deficiency of both vital energy and vital essence and having symptoms of weak mentality, fatigue, tinnitus, poor memory, palpitation, shortness for breath, dizziness, night sweat, insomnia.

3.3 Functional Food of Anti-aging

Functional foods of anti-aging is a food that has one or more compounds with biochemical and physiological functions beneficial to human health, which may help prevent age-related dysfunctions and diseases by modulating certain biological mechanisms in the body[22]. With the improvement of people's living condition, Chi-

nese people are more concerning about life quality, which developes a huge market for the functional food. Flavonoids, grape/wine polyphenols, vitamin E, chlorophyllin and other phenols can protect polyunsaturated fatty acids found in membranes from oxidation, avoiding mitochondrial and other biomembrane disruptions[23]. Other functional components of food that may prevent or reverse mitochondrial damage are coenzyme Q10 (ubiquinone), L-carnitine, lipoic acid, nicotinamide and carnosine[22].

Rather than increasing life span, antioxidants' benefits are related to the control of free radicals that negatively influence healthy aging. Antioxidants are thought to induce antioxidant gene expression, to protect low density lipoprotein (LDL) cholesterol from oxidation and provide antiapoptotic protection of the liver, brain and heart[24]. Chinese researchers are actively seeking natural compounds in foods that have the same oxidation-inhibiting properties. Walnut polyphenols, phenolics and alpha tocopherol in extra-virgin olive oil, ω-3 fatty acids and selenium in nuts, lycopene in tomato-based foods, catechin-rich tea, and soy isoflavones all have shown some inverse relationship to risk factors for aging related chronic disease[22]. It is also reported that antioxidants such as tocopherols, carotenoids, green tea polyphenols, and phytoestrogens decrease oxidative cell injuries and inflammatory reactions, improving brain health[25]. In our studies, we find that both astaxanthin and *Musca demestica larvas*' powder can prevent the decrease of antioxidant enzyme activities in serum. Meanwhile, they can also down-regulate the D-gal and jet lag induced expression of antioxidant genes, aging biomarker β-galactosidase and *Klotho* genes. All these results imply that both functional compounds have anti-oxidative and anti-aging capabilities.

Chinese medicinal cuisine is unique in the world, which can be stretched back to countless generations. Based on TCM, it combines herbal ingredients with traditional culinary materials to produce delicious food with health restoring qualities. The combination of herbal cures and food stuffs not only hide nasty flavors but also reinforces the medicinal effects. When cooking medicinal food, there are variety of fine materials to be chosen, and each material has its own unique flavor. According to their different functions, medicinal cuisine is classified into four categories: health-protection cuisine, prevention cuisine, healing cuisine and therapeutic cuisine. Health-protection cuisine refers to nutritional food corresponding to maintain health. A soup of angelica and carp can tonify Qi and blood. Ginseng congee can give more strength. Prevention cuisine builds resistance to potential ailments. Mung bean soup is considered to guard against heat stroke in summer. Lotus seeds, lily, yam, chestnuts, and pears can assist in preventing dryness in autumn and strengthening resistance to cold in winter. Healing cuisine is a medicinal food for rehabilitation after severe illness. Broiled sheep's heart with rose or braised mutton with angelica will help to rebuild a healthy constitution. Therapeutic cuisine aims at the specific pathology. Fried potatoes with vinegar can invigorate the stomach and restrain hypertension and carp soup with Tuckahoe may enrich the strength of plasma albumen to help reduce swelling.

4. Concluding Remarks

Indeed, the Chinese and their incessant pursuit to find the perfect age-defying product have been present since the earliest times, and remains an important thrust among today's generation. It starts with the Chinese, and yet it is a global concern now. Anti-aging strategies have evolved and yet these efforts remain deeply rooted in ancient Chinese concepts, discoveries and inventions. As a result of a variety of anti-aging research projects, this area of medicine has since flourished. The current focus of anti-aging studies has moved from merely preventing or delaying the onset of aging to the greater objective of stopping the aging process itself. Anti-aging products and treatments include nutrition plans, exercise regimens, skin care, hormone replacements, vitamins, herbs, and supplements. These products do not necessarily lengthen a person's lifespan, but they can help reduce and erase the outward signs of aging. On the other hand, the part of the popula-

tion over 65 who have access to "anti-aging medicine" represents however another challenge to experimental and clinical gerontology, which needs to be comprehensively, systematically, carefully researched.

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Metabolism and Anti-Aging by Caloric Restriction

Rozalyn M. Anderson*

A primary challenge in the study of aging arises from the biological complexity of the aging process itself. Over 75 years ago, a deceptively simple approach of reducing calorie intake proved to delay aging and the onset of age-associated disease in mice. Since then, caloric restriction (CR) without malnutrition has been shown to be the most robust and consistent intervention that improves healthspan and survival in diverse species. The goal of CR research is not to promote it as a lifestyle option, rather it is to use it as a tool by which we can gain novel insights into the aging process and the underlying physiological changes that lead to increased disease vulnerability with age.

Overt phenotypes of CR include improved glucoregulatory function, reduced adiposity, and the preservation of mitochondrial function. The inverse linear relationship between calorie intake and lifespan extension suggests that regulators of energy metabolism are important in CR's actions. We propose that CR induces an altered state of energy metabolism that promotes health and longevity. A key aspect in setting this altered state lies in the impact of CR on white adipose tissue metabolism and systemic signaling. Our studies in mice and monkeys indicate that nutrient sensitive regulators are involved mechanistically in the anti-aging regimen of CR and suggest that they may be effective targets for treatment of multiple age-associated diseases and disorders.

Introduction

The impact of CR in extending lifespan and delaying the onset of age-associated disease in mammals was first described in 1935 [1]. Despite the intervening decades, the mechanisms by which CR promotes longevity and health remain unknown. Aging itself is the most significant risk factor for a range of diseases including cancer, cardiovascular disease and diabetes [2]. Elucidation of the mechanisms of CR will provide crucial leads for understanding the aging process and will identify novel targets for disease prevention. The potential to radically improve our understanding of disease vulnerability as a function of age has encouraged increasing numbers of investigators to study CR's mode of action. Since the 1980s, the annual number of CR publications has grown dramatically, driven in part by the rapid expansion of studies in short-lived species including yeast, worms and flies [3]. The fact that CR can delay aging in diverse species suggests that it impacts a highly conserved aspect of aging. As outlined below, defects in mitochondrial energy metabolism are a common aging phenotype and may be the link between aging and age-associated disease vulnerability.

A decline in mitochondrial function is a hallmark of aging observed in multiple species, and in mammals in multiple tissues. Initial comparisons of gene expression profiles from worms and flies revealed that there are common patterns of age related changes [4]. It turns out that the age-associated decline in expression of genes involved in mitochondrial energy metabolism is highly conserved. A comparison of mouse and human transcriptional data reveals that this same group of genes declines with age in mammalian tissue [5, 6]. At the cellular and

* University of Wisconsin Madison, Department of Medicine, School of Medicine and Public Health; & Geriatric Research Education and Clinical Center, William S. Middleton Memorial Veterans Hospital, Madison, Wisconsin, USA.

tissue levels, mitochondrial dysfunction impacts not only energy availability, but also the regulation and integration of metabolism. Mitochondrial function declines with age in skeletal muscle in humans [7], and increases in intramuscular triglycerides and lipid deposition due to mitochondrial insufficiency correlate with loss of insulin sensitivity [8]. In addition, there is evidence for a mitochondrial component in neurodegenerative disorders; these include Parkinson's disease [9], Alzheimer's disease [10] and Huntington's disease [11]. The coincidence of mitochondrial dysfunction in these distinct disorders [12] suggests that mitochondrial efficiency is important in maintaining neural function and plasticity [13]. These findings raise the possibility that age-associated declines in the nervous system may also have a basis in mitochondrial dysfunction. In heart, deregulation of mitochondrial energy metabolism is a shared phenotype of aging and disease [14]. Taken together, these findings indicate that metabolic dysfunction is a conserved component of aging across species, and among mammals, metabolic dysfunction is a common aspect of diseases that are associated with aging.

Mechanisms of Caloric Restriction

CR induces an active response

For some time the consensus view of anti-aging by CR described a passive mechanism where normal aging was occurring but at a slower rate. In the early 2000's, studies in yeast suggested that CR was an active process [15]. In this model, CR specifically induces a longevity program that is linked to the regulation of metabolism. We can now apply this more recent view of CR to classic mouse studies conducted in the 1980's. As food intake is lowered (while avoiding malnutrition), both average and maximal lifespan of the mice increase [16]. The inverse linear relationship between calorie intake and lifespan suggests that energy utilization and energy production are at central to the mechanisms of CR. Taking insight from the yeast studies, we have proposed that the response to a change in energy availability is the induction of an altered metabolic program that delays aging and prevents disease vulnerability [17].

Mitochondrial energy metabolism

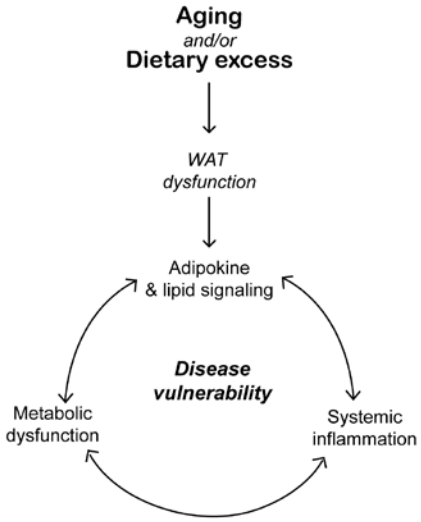
We have examined transcriptional changes with age and with CR in multiple tissues and find shifts in the expression of genes encoding proteins involved in energy metabolism to be a prominent feature of CR in mice. Indeed, alterations in mitochondrial energy metabolism are observed in multiple species on CR [17], including humans [18]. Extrapolating from these studies, it stands to reason that nutrient sensitive factors would be prime candidates in eliciting the response to a change in energy availability. We recently reported a novel mechanism for mitochondrial adaptation in response to oxidative stress that also plays a role in CR [19]. This pathway converges on the nuclear receptor transcriptional coactivator PGC-1α. PGC-1α is a key regulator of mitochondrial energy metabolism, and its activity can be modified through the nutrient sensitive NAD dependent deacetylase SIRT1 and the nutrient sensitive kinase GSK3β. Taken together, these findings suggest a central role for mitochondrial adaptation in the mechanism of aging retardation by CR.

Over the course of a decade, large amounts of gene expression data have been generated from tissues of aged and CR animals. The traditional approach has been to compare old animals on a control or *ad libitum* diet to age-matched old CR animals and then to compare both to young animals. In these studies age associated changes in gene expression are readily identified, but interpretation of the profiles from CR animals is more complex. Two types of transcriptional changes are observed in old CR animals: first shifts in gene expression opposing age-associated changes, and second, genes that are altered with CR that are independent of aging genes. These latter changes are likely to hold clues as to the mechanisms of CR and may reveal the identity of factors involved in the longevity program. A new approach is to investigate changes in gene expression induced by CR in non-aged animals. Utilizing this design to explore the impact of CR on white adipose tissue turned out to be very revealing, as outlined below.

Adipose-tissue activation

Adipose tissue, far from being an inert storage depot for unused fat, acts as an endocrine organ and is involved in organismal metabolic homeostasis [20, 21]. Adipogenic signaling influences energy balance and inflammation [22] and may contribute to the increase in systemic inflammatory tone observed with age. Indeed, aging is associated with adverse alterations in body fat distribution and deregulated adipose function [23-25]. Further evidence for a role for adipose tissue in systemic homeostasis is gleaned from the consequences of dietary excess and the resulting obesity. Metabolic syndrome is the manifestation of multiple conditions including Type II diabetes, inflammation, hypertension and cardiovascular disease [26]. Importantly, many of these conditions have been previously associated with aging. These findings suggest a causative role for adipose dysfunction in disease vulnerability associated with dietary excess and with aging (**Figure 1**).

Figure 1 Model describing how age or diet-induced adipose tissue dysfunction may contribute to disease vulnerability.



In mice, there is an overt change in body composition with CR. The reduction in total body weight usually reflects the level of CR (i.e., 30% less food lowers body weight by ~30%), but weight loss is not distributed equally among tissues. Proportionally more fat mass is lost in mice, monkeys and humans fed a CR diet (i.e., 30% CR leads to ~70% less fat). Furthermore, CR induces morphological and transcriptional alterations in adipose tissue [27], including a striking up-regulation of metabolic genes. Within this group of genes, a coordinate increase in expression of genes involved in oxidative phosphorylation is detected, consistent with a reprogramming of energy metabolism. Furthermore, the expression of over 50 pro-inflammatory genes is reduced in adipose tissue from CR animals compared to controls [28]. These data suggest that adipose tissue from CR animals is functionally distinct from that of controls and has led us to propose a model where changes in adipose tissue function are central to establishing disease resistance as part of the CR longevity program.

The CR induced shift in metabolism in white adipose tissue provides increased capacity for fatty acid oxidation and permits the mobilization of fat stores without increasing oxidative damage through altered mitochondrial function. Fatty acids that possess signaling function are known as lipokines [29]. These bioactive fatty acids influence systemic inflammation through fatty acid binding proteins and act as natural ligands for the nuclear receptor family of transcription factors [30]. Adipose tissue is the major source for circulating fatty acids and alterations in adipose tissue metabolism are reflected in plasma fatty acid composition [29]. This relationship between adipose tissue and serum fatty acid composition suggests a mechanism whereby adipose tissue status influences systemic metabolism. Peroxisome proliferator activated receptor gamma (PPARγ) is a member of the nuclear receptor family that has previously been associated with human metabolic disease [31]. Adipose tissue derived saturated fatty acids mediate inflammation and insulin resistance through PPARγ [32]. In this way, changes in adipose tissue through aging, or dietary excess, or CR, can result in changes in lipokine production and signaling to impact metabolic homeostasis and systemic inflammatory tone.

Insights for Health and Aging

The dietary intervention of CR opposes a broad spectrum of age-associated diseases including cancers and cardiovascular disease. There are two non-exclusive perspectives that

may point the way for future health care consideration. In the first, we view the mechanistic basis for CR's beneficial effects on health and aging and use that information to develop novel targets for disease prevention and treatment. An example would be to identify compounds that can reproduce the effect of CR in activating adipose tissue. The second interpretation stems from the observation that the underlying causes of disease vulnerability are amenable to inhibition through pathways that ordinarily respond to changes in nutritional status. This raises the possibility that disease resistance could be enhanced by means of dietary composition, where key mediators of CR are influenced by nutritional signals provided naturally through the diet.

With an ever-going population of elderly across the world, the emphasis on finding ways to restrain increasing healthcare costs has moved to the forefront. The recent demonstration that CR is effective in improving health and survival in non-human primates argues to a high degree of translatability to human aging [33]. As CR research continues, we can anticipate the development of novel treatments to improve health and delay the onset of age-associated disease.

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In vitro Measurement of Glycemic Response of Foods and Meals Based on Glucose Releasing Rate (GR)

Hideshi Kumai

1. Background of the study

1.1 Blood glucose and disease

Control of blood glucose levels following meals is very important, and is central to a variety of pathologies, including type 2 diabetes mellitus. Moreover, there is mounting evidence which indicates a relationship between blood glucose and both metabolic syndrome and arteriosclerosis, and related diseases, such as coronary heart disease (CHD) and cerebral vascular disorder (CVD), which are both among the major causes of death.

The Glycemic Index (GI) is an in vitro assay method for measurement of the glycemic response to foods, and is a powerful tool to estimate blood glucose levels following meals (1). However, blood sampling is necessary for GI measurement, which results in stress on volunteers, and requires cost and time. Therefore, although many efforts have been put into measuring the GI of foods, there are not comprehensive GI tables reflecting different local and regional foods. Moreover, since the subject (people) are used as the analytical instrument to measure GI, there is not a little variability in the measurements, with a coefficient of variation (CV) of 30%, and sometimes more. Therefore, it is taking time to familiarize consumers with the concept of GI.

1.2 ILSI Japan and GR

This being the situation, ILSI Japan Carbohydrate Task Force has launched a project called "In vitro Measurement of Glycemic Response of Foods and Meals Based on Glucose Releasing Rate", and we also call this technique GR measurement, or GR method. We discussed the methodology of GR measurement, and issued a Basic Research Report in February 2005 (2). We concluded that GR measurement should be simple, low cost, accurate and reproducible. Moreover, we aimed to measure not only individual foods, but meal portions, such as set meals and bentos (boxed lunches) as well. Also, we regard GR of foods in development is important, as well as foods in the market. We conducted a 3-year research project, in collaboration with National Food Research Institute (NFRI), Japan, for the establishment of GR measurement from April 2005 to March 2008.

2. Research and development of GR

2.1 Previous research and basic design of GR

Previous research on in vitro measurement of glycemic response had been conducted by Granfeldt *et al*. (1992) (3,4) and was called the Hydrolysis Index (HI) method and by Englyst *et al.* (1999) (5,6) and was called the Rapidly Available Glucose (RAG) method. However, these methods were not enough evaluated (7). After studying both methods, we optimized the conditions for each digestion step, including crushing food and enzyme reaction in vitro, in light of ease of handling and stability of the results. Each digestion process, namely, the reaction in the oral cavity, stomach, duodenum and small intestine (Fig. 1) were mimicked and replaced by a meat grinder or mincer, a pepsin reaction, an α-amylase reaction and an α-glucosidase reaction, respectively.

* Fermentation Technology Group, Ingredient Development Department
Food Technology Research Laboratories, R&D Division, Meiji Co., Ltd.

2.2 Study of individual digestion steps

2.2.1 α-Amylase reaction

Figure 2 showed the typical example of the time-course of α-amylase digestion of starchy foods. And reaction velocity of digestion, or glucose releasing, obviously reflected the GI of the foods well. We examined the chemical properties of α-amylase, such as enzymatic activity, stability, or optimum pH, and we were able to include this reaction in a GR measurement.

Figure 1 Digestion Process. Strategy for GR measurement

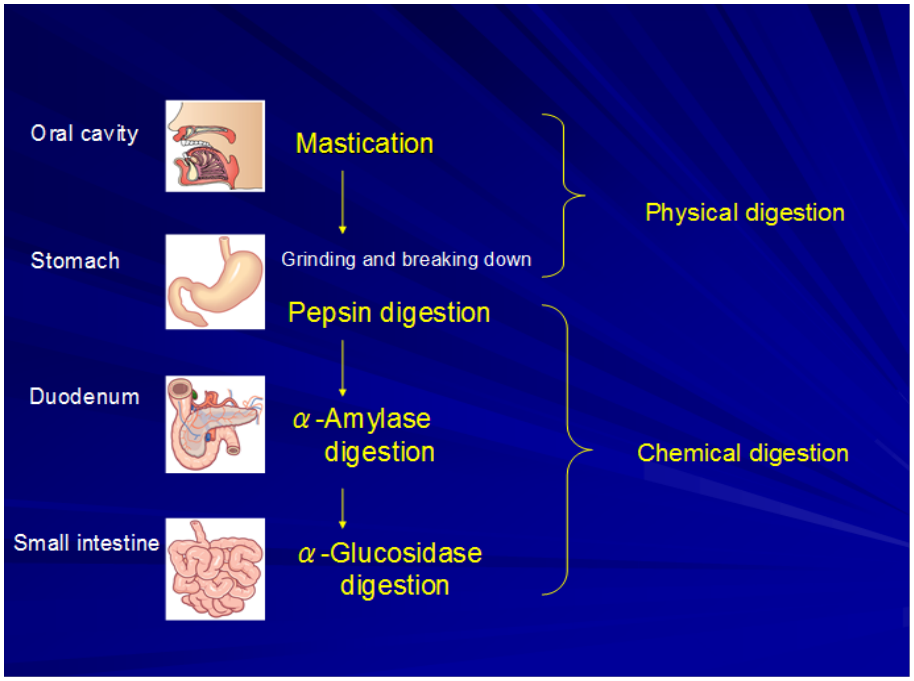


Figure 2 α-Amylase Reaction of Starchy foods.
This in vitro data reflects differences of GI in starchy foods.

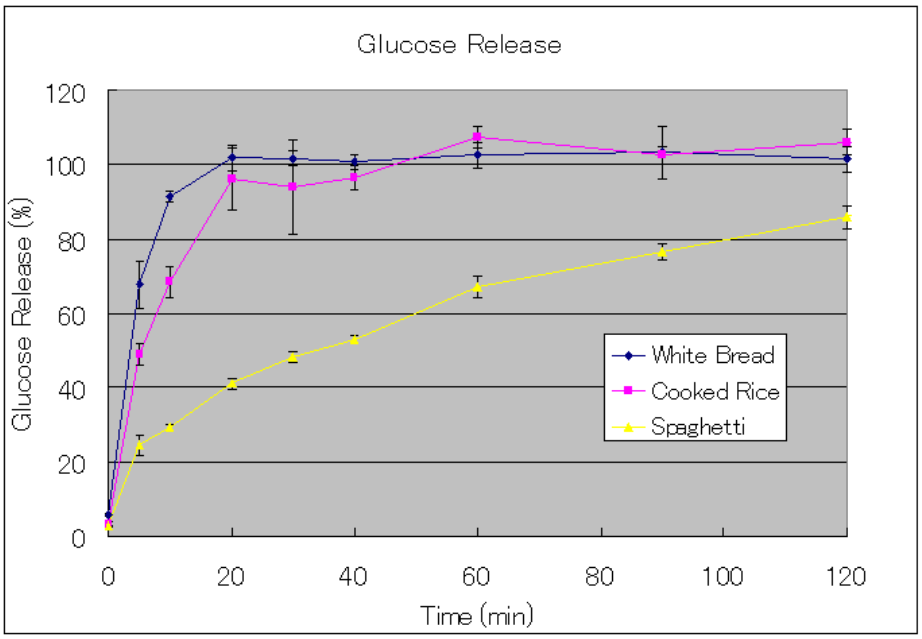


Figure 3 α-Glucosidase activities of RIA (Rat Intestinal Acetone powder)

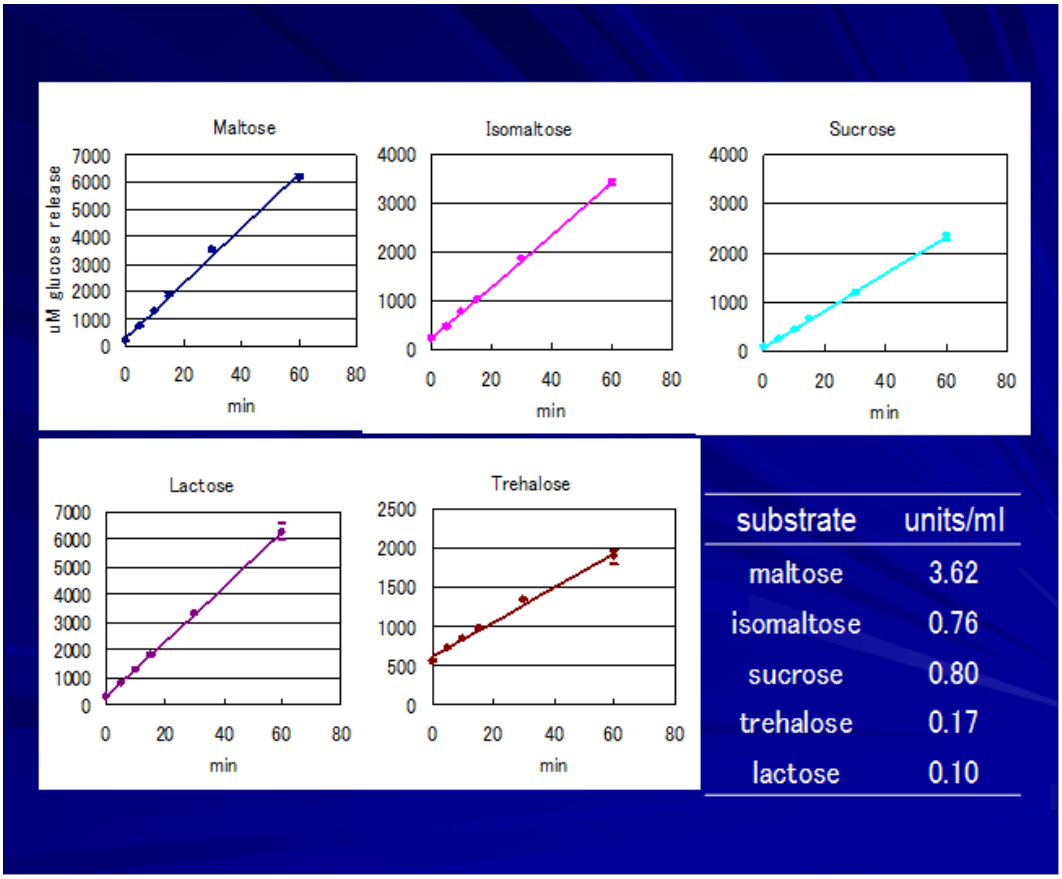
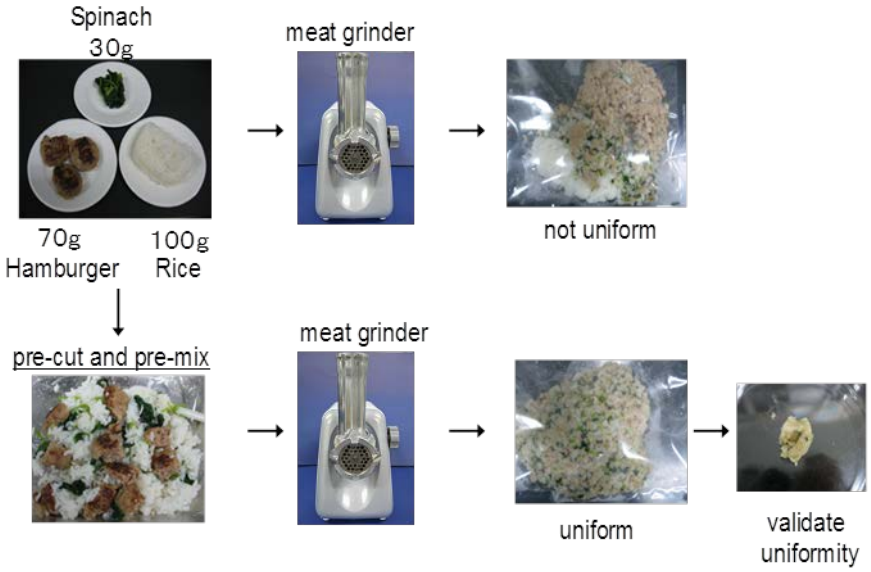


Figure 4 Meat grinder and homogenizer
(a) Meat grinder (MK-GL20W National), (b) Homogenizer (Ermex)

Meat grinder is used for grinding meet, and other foods or ingredients as well. When a food is put into meat grinder from the top of it, it is forced by the motor, then cut by the inner blades and gets out. Homogenizer is like stomacher. When a food is put into plastic bag with water and set in homogenizer, it is broken down.

Figure 5 Physical digestion of 'hamburger steak meal'



2.2.2 α-Glucosidase reaction

We searched for the α-glucosidase enzyme which is available for GR measurement.

There were many enzymes which digest maltose or sucrose, but almost all of them were from bacteria, and their digestion mechanisms are quite different from that of human's (8). Among them, we found Rat Intestinal Acetone powder, or RIA which derived from rat and it is also used for screening of α-glucosidase inhibitors of human (8,9). We examined α- glucosidase activities of RIA, and found RIA has Maltase, Isomaltase, Sucrase, Lactase and Trehalase activities (Figure 3). Activities itself were not so strong, but we managed to include RIA as a step of GR measurement.

2.2.3 Physical digestion (mastication)

In the preliminary study, we recognized the importance of physical digestion, as well as chemical digestions. So, we tried using several devices to break down the foods and the meals. Among them we selected two best devices, which were meat grinder (Figure 4 (a)) and homogenizer (Figure 4 (b)), and we compared the availability and performance of them.

The advantage of meat grinder is simple and easy. It costs about $200. And its methodology is established and supported by research report (10). Moreover, it is also adaptable to variable amount of ingredients. So, we concluded the meat grinder is suitable for crushing of meals. On the other hand, the advantage of homogenizer is that it is adaptable to fiber rich foods, such as vegetables. And it is also adaptable to high water content foods, such as fruits. Moreover, the output is always the same as the input. So, we concluded the homogenizer is suitable for crushing foods. By comprehensive judgment, we chose meat grinder as physical digestion device in the GR measurement, mainly because it is adaptable to meals.

2.2.4 Pretreatment of physical digestion

In order to study the availability of meat grinder to measure the GR of a meal, we prepared the 'Hamburger steak meal' model, which includes 100g of cooked rice, 70g of hamburger and 30g of spinach (Figure 5). When we put the meal, or 3 foods, into meat grinder separately, output of the meal was not uniform, and GR varied and therefore was not measured. Therefore, we tried to improve and develop the method. After we examined the several pre-treatment, post-treatment or co-treatment of the meal, we found that pre-cut and pre-mix was the effective way to prepare the uniform mixture.

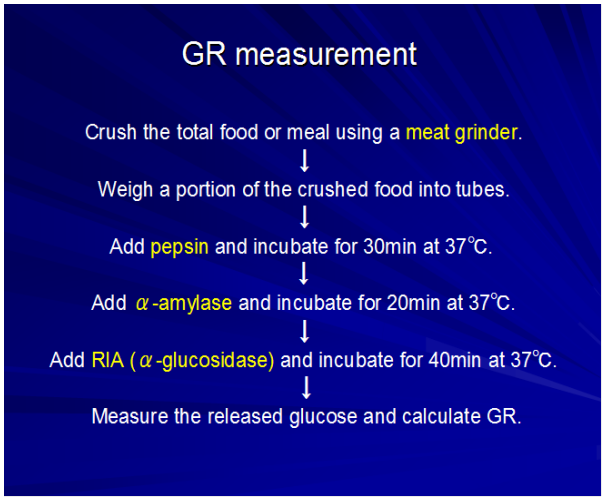
When we pre-cut and pre-mix the meal before putting into meat grinder, we were easily able to obtain the uniform mixture. And uniformity was validated by visualized image,

content of carbohydrate and GR. These three parameters were always constant wherever the sample was picked up. Therefore, we concluded that by using meat grinder and the pre-treatment, we can measure the GR of meals, as well as foods.

2.3 GR method and cost of it

GR measurement was designed by putting together the each reaction we examined and refining the overall process. The enzyme dosage to the amount of carbohydrate and the volume were considered at each reaction. Figure 6 showed the outline of the GR measurement. We are easily able to note the low-cost of the GR measurement, which costs within a dollar per sample once you get meat grinder which is about $200.

Figure 6 Outline of GR measurement



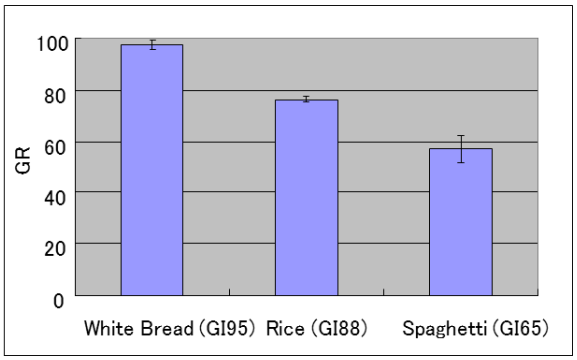
3. Results of GR measurement

3.1 GR of foods and meals

By using the GR measurement, we measured the GR of the starchy foods (Figure 7). As typical examples of high GI, middle GI and low GI starchy food, we selected white bread, cooked rice and spaghetti, respectively. We noted the good correlation between GI and GR. Therefore, it shows a possibility that GR measurement is able to substitute for GI measurement as for starchy foods.

We also measured the GR of several other foods and meals (Figure 8). We measured rye bread and brown rice as typical low GI foods, and gruel rice. Moreover, we measured noodles such as ramen, udon and soba and also glutinous brown rice. And we also measured the 'kit meals' such as chicken curry and hamburger meal.

Figure 7 GR of starchy foods
GR of the foods is related with GI.



We also measured the GR of the set meals at cafeteria accompanying NFRI. On September 13th in 2007, A set meal was grilled sliced meat (Figure 9(a)), B set meal was fried fish and vegetables (Figure 9(b)) and Variety meal was omelet and rice (Figure 9(c)). Each meal was accompanied with miso soup and pickled vegetables. GR of each meal was measured, which was 76, 73 and 76, and we learned that the GR of B set meal was a little lower (Figure 9(d)).

3.2 Reproducibility of GR

We examine the reproducibility of GR measurement using the standard test meal. The standard test meal is E460F18, which is energy and fat-adjusted set meal and is a hospital diet. As for the simultaneous reproducibility of the GR measurement, CV, or coefficient of variation at 3 each test was 1.6%, 0.6% and 2.5%, respectively (Figure 10). Moreover, as showed above, CV was within 5% at almost all foods or meals. As for the daily reproducibility of the GR measurement, the average GR of these 3 tests were 92.6, 92.2 and 90.4. Therefore, CV of the 3 daily tests was 1.3% (Figure 10). These figures are almost the same as that of other biochemical experiments in liquid phase. From the above-mentioned results, we concluded that the reproducibility of GR measurement is satisfactory, and it also satisfied one of our goals we planned i.e. accurate and reproducible.

Figure 8 GR of foods and meals

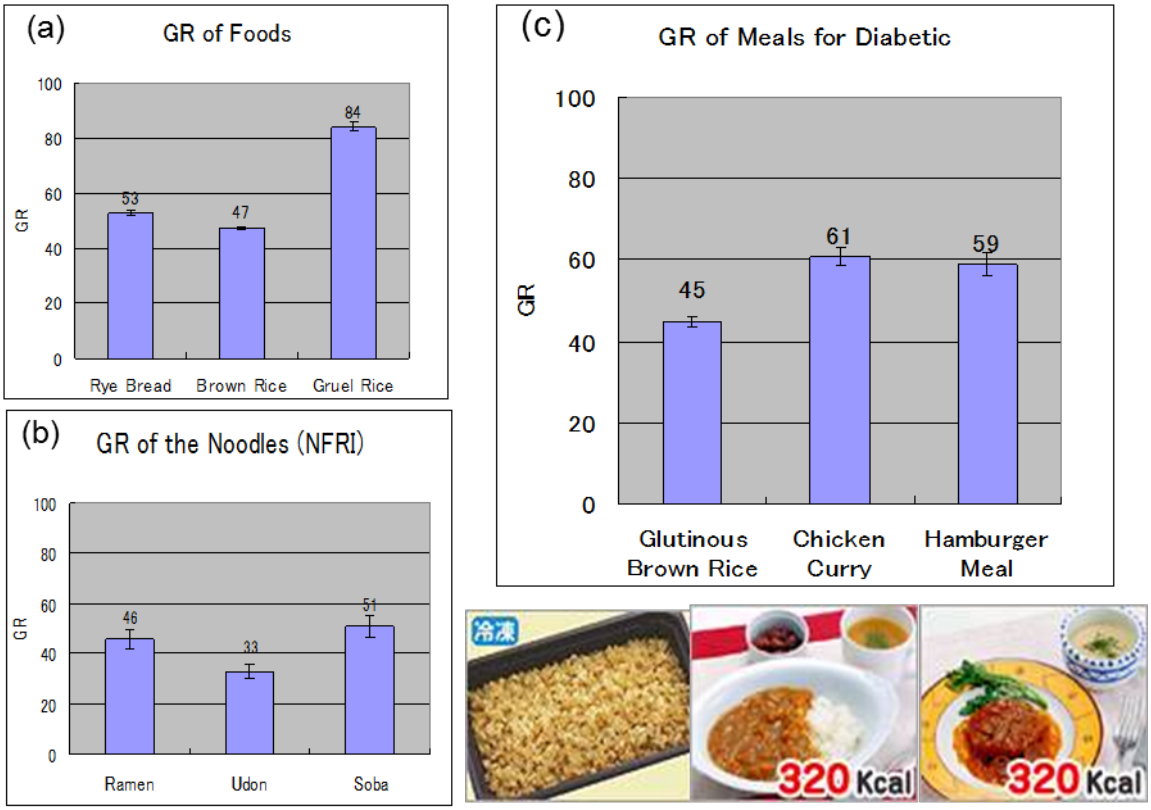


Figure 9 GR of the set meal at NFRI

(a) A set meal (b) B set meal (c) Variety

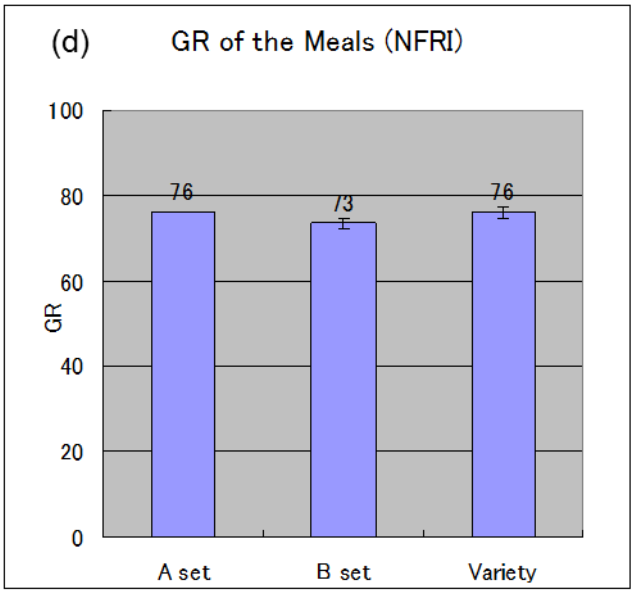
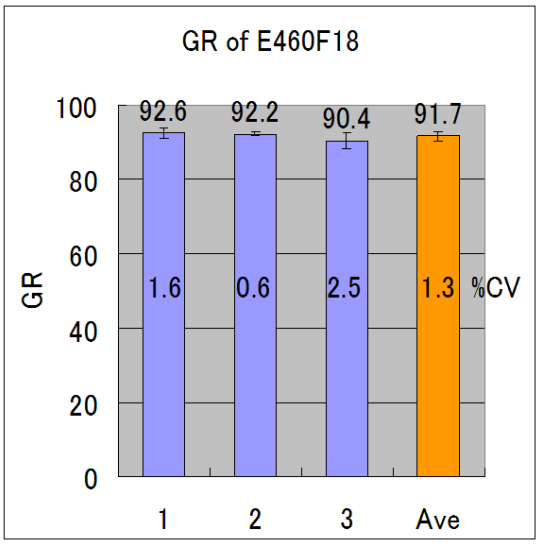


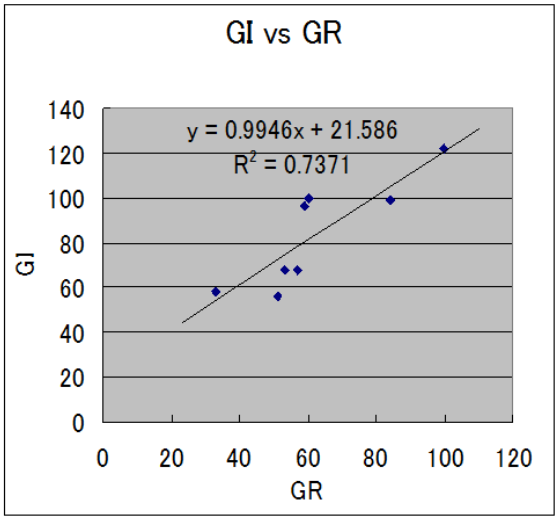
Figure 10 Reproducibility of the GR



3.3 Correlation between GI and GR

In order to investigate the reflection of the blood glucose after an intake of foods to GR, we compared the GI (11,12) and GR of the foods. As showed in Figure 11, there is a good correlation between GI and GR. Therefore, GR will, at least, be able to substitute for GI measurement. We noted some discrepancy between GI and GR, which may be derived from the difference of the measurements or the inaccuracies of GI.

Figure 11 Correlation between GI and GR



3.4 Review of GR measurement

We summarized the feature of the GR measurement. GR measurement is (1)simple and able to be conducted in typical laboratories, (2)low cost, (3)adaptable to meals, (4)well correlated with GI and (5)accurate and consistent. Consequently, we concluded that the GR measurement we developed achieved the objectives we had planned and published in the paper.

4. Definition of 'available carbohydrate'

4.1 Why available carbohydrate?

Available carbohydrate is one of the most likely candidates of being denominator of GR. Therefore, determination of available carbohydrate content in food is important. Nevertheless, the determination by the composition table is virtually impossible

For almost all foods, the percentage of carbohydrate is described on Standard Tables of Food Composition in Japan 2010 (13). However, the percentage of carbohydrate varies among products, and their variation is sometimes larger than SD of GR. Therefore, 'Standard Tables' is not available as the content rate of available carbohydrate of the food. And for each food product, 'Nutrition Composition' is labeled on the package of it. However, carbohydrate is labeled as Tansuikabutu in a case, which includes dietary fiber, but it is labeled as Toushitu in another case, which does not include dietary fiber. Therefore, 'Nutritional Composition' is not always available. Consequently, we have to determine the available carbohydrate content in another way.

From another point of view, starch gains 10% weight when it is hydrolyzed, or digested. So theoretically, GR of starch is nearly 110, and GR of maltose is about 105, in other word, more than 100. This is not allowed because GR of glucose is defined as 100 and maximum.

And finally, and most importantly, at the research and development of the healthy food, we should exclude the false, or sham low GR food which contains much of non-available starch.

For the reasons stated above, we came to conclude that the definition of available carbohydrate is crucial, and denominator of GR should be concretely defined.

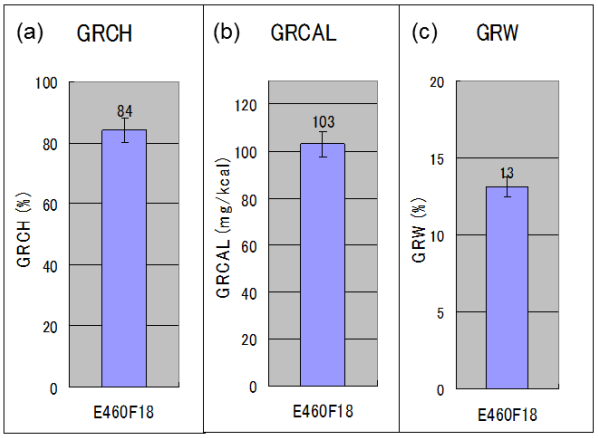
4.2 Denominator of GR

In the discussion at ILSI Japan and NFRI,

we discussed and chose some of the denominator of GR. And we found that 3 denominators were available, and we proposed GR per carbohydrate, GR per calorie and GR per weight (Figure 12). And we noted that each ratio of Glucose Release to denominator has some important meaning of the food property.

GR per weight is obtained in any case. GR per calorie is obtained when 'Nutritional Composition' is labeled. On the contrary, as showed above, GR per carbohydrate is not obtained in most case. However, it is no doubt that GR per carbohydrate is the best way to express the property of the foods or meals. So, we tried to make a definition of carbohydrate, in this case available carbohydrate, by both experimental and logical way.

Figure 12 Denominators of the GR
(a) GR per carbohydrate (Nutritional Composition)
(b) GR per calorie (Nutritional Composition)
(c) GR per weight



4.3 Definition of available carbohydrate

Available carbohydrate should be measured (1)in an easy way, (2)based on theory and (3)in every sample, foods or meals. For such situation, we researched and learned the technical term 'non-resistant starch'. Non-resistant starch was experimentally defined in two publications by McCeary in J AOAC Int. 2002 (14,15). Conveniently, this method is almost the same as the GR measurement when α-amylase reaction continues for 16 hours. Therefore, the amount of the non-resistant starch, or glucose release at 16 hours, is obtained by only a minor modification.

And experimentally, this amount did not change when incubation time extended for 2 days or longer, meaning it is the limitation of glucose releasing of the food in intestine.

Taking these findings into consideration, we defined the glucose release at 16 hours as available carbohydrate.

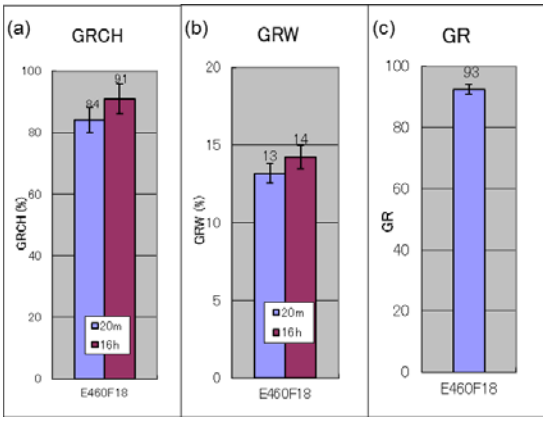
4.4 Calculating GR

Figure 13 shows the concrete example for calculating GR of the meal. In this case, using Nutrition Composition, we measure the glucose release per carbohydrate at 20 minutes (Gr20m) and at 16 hours (Gr16h) (Figure 13(a)). GR is defined as a ratio of rapidly available carbohydrate to available carbohydrate, therefore GR is derived from Gr20m divided by Gr16h, or 84/91, which is 93 (Figure 13(c)). When Nutritional Composition is not labeled, using based on GR per weight (Figure 13(b)), which is always available, the same GR is deduced.

Gr16h at GRCH (91%) is available carbohydrate, and 100% is the total carbohydrate, so the remaining 9% indicates the dietary fiber or non-available starch of the meal. Moreover, CV of the measurement gets smaller, because Gr20m and Gr16h are deduced from the sampling of the same vessel.

Altogether, available carbohydrate is defined experimentally and theoretically in an easy way in every samples, foods or meals. Moreover, we succeeded to achieve more accurate measurement, exclude the effect of weight gaining of hydrolyzed starch and eliminate false low GR foods.

Figure 13 GR calculation
(a) GR per carbohydrate (Nutritional Composition)
(b) GR per weight (c) GR
GR is derived from Gr20m/Gr16h. Gr20m: Glucose release at 20 min. Gr16h: Glucose release at 16hours.



5. Future prospects for GR

5.1 GR is an inherent property of foods?

5.1.1 Feature of GR

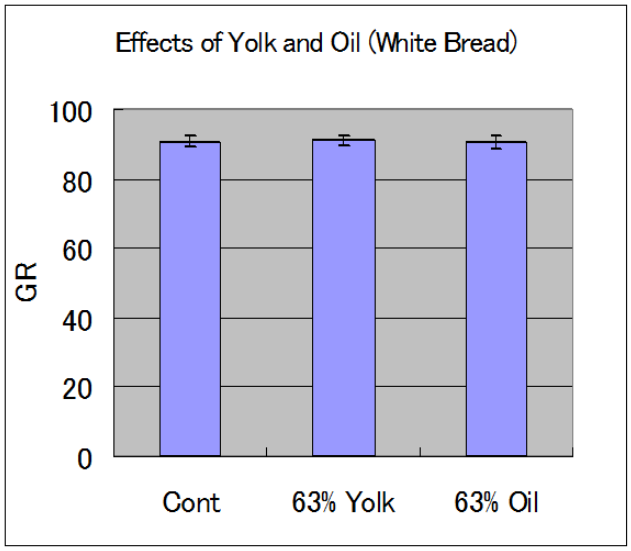
GR measurement is an in vitro assay, and not an in vivo one, therefore, GR reflects mechanical conversion as physical factor and enzymatic conversion as chemical factor. However, GR does not reflect incorporation of glucose of both intestinal membrane and blood vessel membrane nor gastrointestinal hormone as physiological factor. This is the most important feature of the GR measurement. Consequently, GR *may* be an inherent property of the foods.

5.1.2 Rigidity of GR measurement

We studied the rigidity of GR measurement because foods or meals are mixture, which include unexpected ingredients. We examined and elicited the following conclusions. GR measurement is stable between pH 6 and 8, little influenced by salt and not affected by adding even large amount of non-starchy food, such as fat and protein. Figure 14 showed the GR of white bread adding 63%, technically maximum percentage, of yolk or salad oil. No effect was observed of addition of yolk or salad oil. Consequently, GR is *probably* an inherent property of the foods, because their GRs are not supposed to be affected by each other.

Figure 14 GR of white bread in the presence of yolk or salad oil

GRs are not affected by adding 63% of yolk or oil.

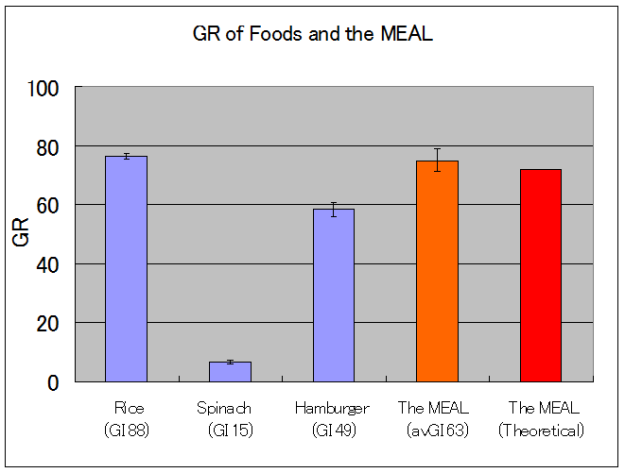


5.1.3 Influence of other foods to GR

If GR is really an inherent property of the foods, we are able to add up the GR, or calculate the average GR in case of plural foods. By using the 'hamburger steak meal' as showed above, we examined the GR of mixture of the foods (Figure 15). The light blue bars indicate the individual GR of the foods, which are rice, hamburger and spinach. And the orange bar indicates the measured GR of the meal. The red bar indicates the theoretical GR of the meal, which is elicited from carbohydrate-weighted average of GR of these three foods. The measured GR of the meal is quite similar to theoretical GR. Therefore, the GR of the meal is likely able to be obtained from the GRs of the composed foods. Consequently, GR *must* be an inherent property of the foods.

Figure 15 GR of the 'hamburger meal'

Measured GR of the Meal is quite similar to theoretically GR derived from composed foods.



5.1.4 Glucose Releasing Load (GRL)

And similarly, if GR is an inherent property of the foods, Gr20m, or the numerator of GR, is able to be added up, and the total amount of Gr20ms is Glucose Releasing Load (GRL). And GRL means the total rapidly available intestine-digested carbohydrate, which is to be converted to glucose in human body.

5.2 Future prospects for GR

If GR is an inherent property of foods, 'carbohydrate weighted average GR' and GRL of foods or meals are to be obtained. Therefore, without measurement, we are easily able to

calculate the GR of many complex foods, such as set meals when GRs of the composing foods are already known. Moreover, we are able to calculate the GR of any varied portion of meals, such as there are large servings or leftovers.

GRL, or total rapidly available carbohydrate of the foods and meals, will be the good indicator of glucose supply to the blood and is actually converted to blood glucose. Therefore, GR will provide the information concerning not only the quality but the quantity of carbohydrate in foods. As Prof. Brand-Miller, one of the leading scientists concerning and promoting GI, admitted (16), Glycemic Load is not so important, most likely because GI cannot be added up, as foods interfere with each other. On the other hand, GR is able to be added up and provide an important meaning concerning human health. Including these points of view, when the concept and technique for GR spreads worldwide, studies relating GR to disease prevention will be conducted and this relationship will be clearer. GR will be a powerful tool for developing healthy or 'functional' foods and information communicated to customers through labeling. In this way, we expect GR will contribute to global health.

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Moreover, wishing to thank the audience here in the University of Tokyo, Yayoi Auditorium, Ichijo Hall for enthusiastic concentration.

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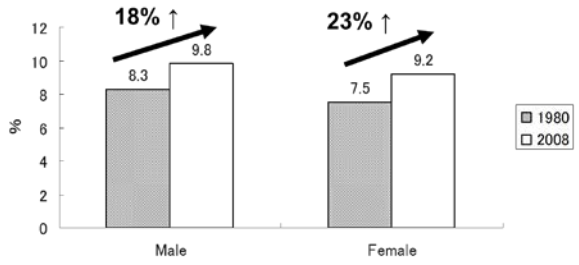
Carbohydrates Task Force; Toward Practical Application of the GR Method

Yukiko Nakanishi*

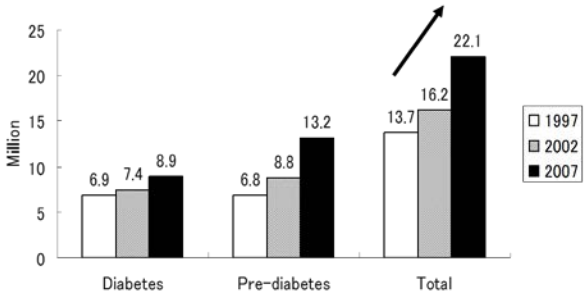
In 2008, global age-standardized adult diabetes prevalence was 9.8% in men and 9.2% in women, up from 8.3% and 7.5% in 1980. The number of people with diabetes increased from 153 million in 1980, to 347 million in 2008[1)] (Figure 1). According to the National Health and Nutrition Survey 2007 conducted in Japan, the numbers of people strongly suspected of having diabetes and of people with possibilities of having diabetes were 8.9 million and 13.2 million, respectively (Figure 2). To prevent from lifestyle-related diseases, controlling of postprandial blood glucose level is crucial. For the food products development, it is necessary to develop a new convenient method to compare the effects of various foods or meals intake on the postprandial glucose response.

Figure 1 Diabetes on Upswing Worldwide



ILSI Japan Carbohydrate Task Force has launched a project called “In vitro Measurement of Glycemic Response of Foods and Meals Based on Glucose Releasing Rate”. As a result of a 3-year research project, in collaboration with National Food Research Institute, Japan, ILSI Japan established a prototype of GR measurement. It was consisted of 3-steps like as the reaction in the oral cavity, stomach, duodenum and small intestine. Toward practical application of GR method, it should be a simplified method which don't need high-specific facilities and by which people with limited experience can measure accurately. The results must be independently replicated in different laboratories. Now we have been conducting the multicenter studies to examine within-run reproducibility and inter-group reproducibility and revising the protocol of GR measurement.

Figure 2 Diabetes affects 22.1 million people of all ages in Japan



Until now, we have conducted a multicenter study twice. For the first trial, 11 laboratories (9 companies and 2 universities) tested the GR method with GR-test Kits which were prepared by a laboratory. As test meals, three meals were selected: commercially-prepared and energy-controlled meal, commercially available liquid meal, and maltose solution as a control meal. The reproducibility coefficient of variance (CV) on the GR measurement was within 10% (Table 1) and the GR method was expected to be more accurate by revising the sampling procedure and selecting other preservative agents. It is crucial to prepare the document of the protocol as well.

* Department of Food Design, College of Nutrition, Koshien University

Table 1 Glucose concentrations and GR values from 46 samples

	Std Rapid	Std Whole	LiqM Rapid	LiqM Whole	Mal Rapid	Mal Whole	Std GR	LiqM GR	Mal GR
MEAN	16.4	20.2	22.7	24.1	26.5	26.7	80.7	95.1	99.5
SD	3.4	3.6	4.0	4.1	4.8	4.7	7.1	5.1	6.1
Meals	46	46	46	46	46	46	46	46	46
MIN	7.4	10.1	11.2	11.9	13.1	13.2	65.1	82.6	88.8
MAX	26.1	29.3	30.5	33	36.2	35.8	98.5	115.3	125.4
CV	20.9	17.9	17.5	17.0	18.0	17.6	8.8	5.4	6.1

Table 2 Glucose concentrations and GR values from 40 samples

	Std20	StdT	Mal20	MalT	StdGR	MalGR	Ajusted StdGR
Mean	13.2	19.3	26.1	25.9	68.4	100.8	68.0
SD	3.5	3.7	4.7	4.4	10.8	6.2	10.7
No	40	40	40	40	40	40	40
Min	5.3	8.2	11.7	12.3	45.0	91.8	43.1
Max	22.3	24.9	34.1	33.2	90.3	117.0	83.9
CV	26.3	19.3	18.0	17.0	15.8	6.1	15.7

For the second trial, 10 laboratories (7 companies and 3 universities) tested the revised GR method with two types of test meals, commercial energy-controlled meal and maltose solution. The CV on the GR measurement was greater compared to the first trial (Table 2). Therefore, we are struggling to re-check the effects of storage and thawing condition on the enzyme activities and to re-select the preservative agent, etc.

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Progress towards the Use of Monsanto's Healthy Omega-3 Fatty Acid Producing Genetically Modified Soy as a Food

Richard S. Wilkes*

Abstract:

It has been widely reported in the literature of the health benefits of long chain polyunsaturated fatty acids (LCPUFA) including the role of omega-3s in heart disease. The omega-3s of particular interest in the prevention of cardiovascular disease include eicosapentaenoic acid (EPA – 20:5) and docosahexaenoic acid (DHA – 22:6)). The primary source of these LCPUFAs is from fatty fish. However, maintaining consumption at sufficient levels in diets which are becoming more westernized is becoming difficult due to the potential of overfishing and contamination concerns. The predominant omega-3 fatty acid in westernized diets is alpha linolenic acid (ALA 18:3n3), which has been reported to have inefficient conversion to EPA. Although consumers have indicated a desire to obtain LCPUFA from food rather than supplements (Natural Marketing Institute, 2008), formulating food using fish oil has proven to be difficult due to flavor and shelf life concerns. As a result, a need exists for another sustainable source of omega-3 fatty acids that can be used in a range of foods and provide the benefits of LCPUFA.

A biotechnology-derived soybean has been developed to produce soybean oil containing stearidonic acid (SDA – 18:4), an intermediate in the metabolic production of EPA from ALA. As a sustainable source of omega-3, it produces more EPA in the body when consumed. Refined SDA soybean oil contains approximately 20% to 30% (wt% of total fatty acids) of SDA, and with less double bonds than EPA and DHA, will provide improved stability to oxidation than fish oils, expanding the potential formulation choices for food companies and consumers. SDA soybean oil may be used in a variety of food products including margarines, mayonnaise, salad dressings, beverages, sauces, snacks and ready to eat foods.

To evaluate the ability of soybean oil containing SDA to impact key cardiovascular markers including the omega-3 index, several clinical nutrition studies have been conducted. The higher the omega-3 index (O3I), which measures the percentage of EPA and DHA levels in red blood cells, cardiovascular disease risk is reduced. In a randomized, placebo-controlled, double blind study with healthy volunteers, volunteers were divided into three groups: the first group received conventional soybean oil, the second group received SDA soybean oil containing 4.2 grams/day of SDA, and the third group received 1 gram/day of EPA. At 12 weeks, supplementation with SDA soybean oil significantly increased the O3I compared to conventional soybean oil. The increase was not significantly different from that obtained after supplementation with EPA. The data confirm previous data from smaller studies using ethyl esters and SDA soybean oil which suggest that consuming SDA significantly increases EPA in red blood cell membranes. The results also suggest that SDA is approximately one third to one fifth as effective as EPA at in-

* Director – Food Applications for Monsanto Company, St. Louis, MO USA.

creasing tissue EPA concentration in humans.

Introduction:

The health benefits of long chain omega-3 fatty acids (n-3 PUFAs) also known as n-3 fatty acids have been reported in the scientific literature (Leaf et al. 2003) including the impact on cardiovascular disease. Consumers have developed increased awareness of the benefits of n-3 fatty acids that has resulted in increased consumption. Although US dietary guidelines have not made specific intake recommendations for n-3 fatty acids, organizations such as WHO, American Heart Association and the American Dietetic Association recommend 250-500mg/day. However, national databases, especially in countries with western diets including EFSA, US NHANES demonstrate that in many areas of the world recommended intake of long chain n-3 fatty acids are not being met. The World Health Organization reports that cardiovascular disease continues to be the primary cause of death globally. Although the sales of n-3 supplements have increased, many challenges exist in formulating food products related to lipid oxidation that result in flavor and shelf life compromises. Finding new sources of n-3 fatty acids that can provide the health benefits of long chain n-3 PUFAs which can be easily incorporated into food will be needed to increase overall n-3 fatty acids consumption. Modified soybeans containing stearidonic acid (SDA, 18:4 (n-3)) may offer the opportunity to increase n-3 fatty acids consumption.

Stearidonic Acid:

The primary omega-3 found in vegetable oils and some nuts is alpha linolenic acid (ALA) (18:3 n-3). Unfortunately, it has been widely reported that ALA conversion into EPA is not very efficient and is rate limited by the delta-6 desaturase enzyme. Stearidonic acid (18:4 n-3) is an intermediate that results from the desaturation of ALA that is more efficiently converted to the more desirable eicosapentaenoic acid (20:5 n-3). By introducing desaturase enzymes, specifically delta-6 and delta-15 desaturases, it is possible to develop a soybean which contains SDA. When oil from the soybean is consumed, the level of EPA in the human body can be increased. Analysis by Akoh and Vazquez (2011) demonstrated the resulting fatty acid composition of the oil and is found in Table 1.

Table 1 Fatty acid profile of SDA soybean oil

Fatty Acid	SDA Soybean Oil Wt % (1)	Commodity Soybean Oil Wt % (2)
Palmitic (16:0)	12.2	11.0
Stearic (18:0)	4.2	4.0
Oleic (18:1 n-9)	15.9	23.0
Linoleic (18:2 n-6)	24.5	54.0
γ-Linolenic (18:3 n-6)	7.2	N.D.
α-Linolenic (18:3 n-3)	10.8	8.0
Stearidonic (18:4 n-3)	23.7	N.D.

(1) From Luis Vazquez and Casimir C. Akoh, University of Georgia, (unpublished data).
(2) Wang 2002

The long term sustainability of sourcing omega-3 polyunsaturated fatty acids exclusively from marine sources is of concern. SDA soybeans provide a more sustainable option that can provide the benefits of EPA when incorporated into foods.

As mentioned, sources of ALA are not efficiently converted to EPA despite the availability in a wide range of oils including soy, rapeseed and flax. These oils, however are easily formulated into food without compromising flavor and shelf life. However, when formulating foods with the more desirable long chain polyunsaturated fatty acids including EPA, challenges due to oxidation and rancid exist when added to food. SDA strikes the right balance by providing an oil that can be added to a range of food products and provides the heart health benefits of EPA.

Nutritional Benefit Studies:

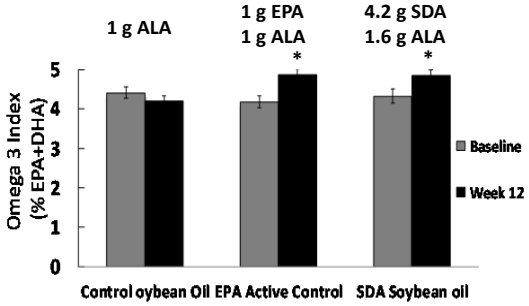
The health benefits of long chain polyunsaturated fatty acids have been widely reported, including the key observations by GISSI-Provenzionne (1999) and in other key studies (Kris-Etherton et al, 2002). Yokoyama et al. (2007) reported that the Japan EPA Lipid Intervention Study confirmed that EPA sup-

plementation alone, without additional DHA, reduced the risk of cardiac events. A program has been implemented that demonstrates the benefit of SDA in the diet.

James et al (2003) reported on a study using ethyl esters that demonstrated SDA could enrich tissues with EPA. In reviewing the results, the relative efficiency compared to EPA was approximately 4:1. In a study completed by Harris et al. (2008), they compared the effects of EPA to a SDA soybean oil containing 20% SDA on erythrocyte EPA + DHA levels (the omega-3 index). The omega-3 index is an emerging marker that can be used to indicate risk of cardiovascular disease (Harris and Von Schacky, 2004). Overweight healthy volunteers were randomized to SDA-SBO (24 ml/day providing ~3.7g SDA) or to regular soybean oil (control group) with or without EPA ethyl esters (~1 g/day) for 16 weeks. A per protocol analysis was conducted on 33 subjects (11 per group). Compared to baseline, average omega-3 index levels increased 19.5% in the SDA group and 25.4% in the EPA group. Relative to EPA, SDA increased red blood cell EPA with about 17% efficiency, demonstrating that SDA enriched soybean oil significantly raised the omega-3 index.

In a more recent study, Lemke et al. (2010) conducted a randomized, placebo-controlled, double blind multicenter study in which 252 overweight subjects were randomly assigned to 1 of 3 treatments for 12 weeks. 1 g encapsulated soybean oil/day plus 14.7 g liquid soybean oil/day to be mixed in food (control group), 1 gram encapsulated EPA/day plus 14.7 g liquid soybean oil/day (EPA group) and 1 gram encapsulated soybean oil/day plus 14.7g liquid SDA enriched liquid soybean oil, providing 4.2 g SDA (SDA group). Subjects consumed treatment oil in exchange for other oils in their diet. The results, shown in Figure 1, show that although the mean baseline omega-3 index and amount of EPA in red blood cells were similar between treatments, after 12 weeks of treatment, values for the omega-3 index for the EPA and SDA groups significantly increased whereas there was no increase in the control group. This increase was a result of an increase in red blood cell EPA levels. The efficiency of SDA relative to EPA was 18:3%, or a ratio of 1:5.5. Adding SDA soybean oil to everyday food products will increase the O3I and the change in O3I is time and dose dependant, consistent with first-order kinetics.

Figure 1 Effect of SDA and EPA on the Omega-3 Index



Mean (±SEM) for per protocol population of 157 subjects
* p<0.001 compared to soy oil control;
SDA and EPA not different p=0.585; ANCOVA

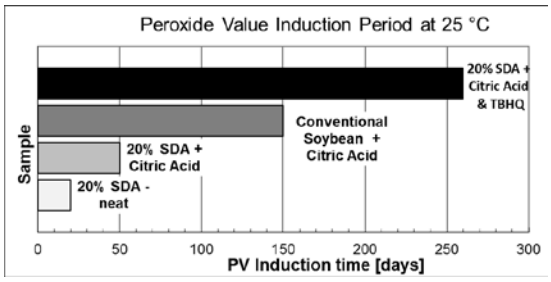
SDA Soybean Oil Use in Food

Oxidative stability is the major challenge in utilizing n-3 fatty acids when formulating foods. As fatty acids increase in the degree of unsaturation, or the number of double bonds, their susceptibility to lipid oxidation increases, making it more difficult to maintain a food's characteristic flavor both initially and throughout the intended shelf life. As a result, Frost & Sullivan recently reported that the growth of omega-3 enrichment in foods has not yet met its potential. Although there has been significant growth in the supplement area, marketing research confirms consumers prefer food to help manage cardiovascular health. SDA soybean oil has improved oxidative stability compared to marine oils and may offer food companies a choice of ingredients to provide the benefits of long chain polyunsaturated fatty acids and maintain product quality and shelf life with good taste.

The impact of oxidation on SDA soybean oil has been evaluated. In one study using the American Oil Chemists Society oven storage test for aging of oils (AOCS, 1997), SDA soybean oil under different antioxidant treatments was evaluated at 25C over an extended

time utilizing a dark oven. Peroxide value (PV) which is a measure of oxidation were monitored throughout the study. The point at which the PV of the oil begins to significantly increase is referred to as the induction period and is an indication of when oil oxidation is occurring and the oil can no longer be used. Results are found in Figure 2. When SDA soybean oil contains only citric acid, which is typical in the edible oil industry, the induction period is prolonged from SDA without any antioxidants, from 20 days to 50 days. When a common antioxidant, tertiary butyl hydroquinone (TBHQ) is added, the induction period is further extended to over 250 days.

Figure 2 Peroxide Value Induction Times of SDA Soybean Oil with Different Antioxidant Treatments

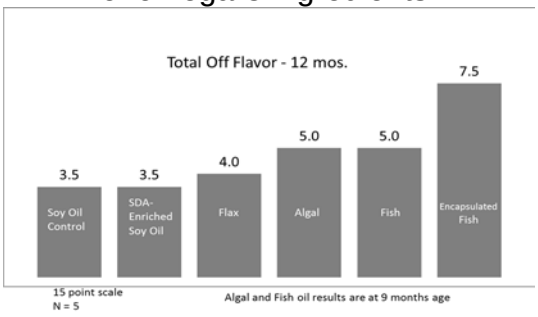


Oils stored at 25C were also evaluated using a trained sensory descriptive panel and compared to commodity soybean oil. At three months age, the overall flavor intensity of the SDA sample and commodity soybean oil were the same, and at very low intensity of 1 out of a 15 point scale. The SDA soybean oil sample did exhibit a low level of off notes, again 1 out of a 15 point scale and below the level of detection by the typical consumer.

With a bland flavor, SDA soybean oil has the potential to be added to a broad range of applications including food emulsions such as margarine/spreads, shortenings, mayonnaise, salad dressings and peanut butter; dairy products including yogurt, nondairy creamers, sour cream, dips and cheese; beverages which can include smoothies, soy milk, fruit juices and drinks; baked products including bread, cookies/crackers, muffins, bagels, pastries, cakes and cereal and energy bars; and prepared foods including soups, sauces, prepared meals. A range of food product prototypes have been prepared to evaluate the impact of SDA soybean oil on flavor, shelf life, and more important, consumer acceptance. Based on past clinical study testing and taking into consideration relative conversion rates and recommendations for both n-3 fatty acids and EPA consumption, a target of 375 mg SDA per serving was used.

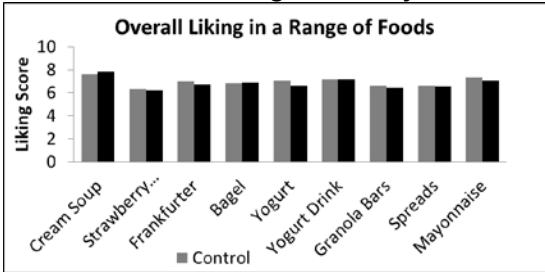
In one example, granola or muesli fruit and nut bars were prepared evaluating SDA soybean oil to other sources of long chain omega-3s. Commodity soybean oil was used as the control. The oil was added to the binder syrup that contained a mixture of sugar and corn syrups, and then added to the cereal and dried fruit mix. The full mixture was sheeted, cut and packed into individual packages and held at room temperature for 12 months. A trained descriptive panel evaluated the bars throughout the shelf life evaluating each bar for key flavor and texture attributes including overall aroma and flavor, sweetness, fruit flavor, oil flavor intensity, off flavor and aftertaste. Figure 3 shows the results of for the off flavor attribute. For the marine and algal samples, the panelists could not taste the samples after 9 months due to the high level of off flavor. The results shown are 9 months age for those samples. The degree of off flavor in the SDA soybean oil sample was the same as for the commodity soybean oil control. The panelists also evaluated the amount of quality change each bar exhibited compared to the original evaluation. The SDA soybean oil bars actually exhibited the least amount of quality change at the end of the 12 month shelf life.

Figure 3 Off flavor intensity of fruit and nut granola bars made with different sources of omega-3 ingredients.



Prototypes of everyday foods containing SDA soybean oil have also included cream soup, strawberry beverage, frankfurters, yogurt, yogurt drink, fruit and nut granola bars, 60% fat margarine type spreads and mayonnaise (Whittinghill and Welsby, 2010). Figure 4 summarizes the result of consumer acceptance testing across a range of food products. In each case, a sample of untrained consumers (n=35 to 60, dependent on individual test) evaluated each food product compared to a control made with commodity soybean oil for overall liking, flavor and aroma utilizing a 9 point hedonic scale with 1 being extremely dislike and 9 being extremely like. All samples were presented to consumers at a typical age they would consume each individual food prototype. The results demonstrate that there were no significant differences in overall liking, indicating equal consumer acceptance of SDA-enriched food products compared to the appropriate control prototype.

Figure 4 Consumer acceptance of a range of foods containing SDA soybean oil



Conclusions:

Despite increasing awareness of the reported health benefits of long chain omega-3 fatty acids, consumer intake continues to be lower than current recommendations. Due to the high level of oxidation found in current sources of long chain omega-3s, concerns with the flavor and shelf life of foods enriched with omega-3 have not been as successful and new sources of these ingredients are needed. Soybeans have successfully been modified to contain 20% stearidonic acid, an intermediate between alpha linolenic acid and EPA. Clinical nutrition studies have demonstrated that when consumed, SDA has the potential of increasing the omega-3 index through increased EPA in red blood cells. With a bland flavor, SDA soybean oil can be added to a range of food products that maintain product quality and shelf life. Consumers have indicated equal acceptance of foods made with SDA soybean oil as with foods with commodity soybean oil. As a result, SDA soybean oil represents a potential choice for food companies to provide consumers with foods containing an omega-3.

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Threshold of Toxicological Concern (TTC) Concept and Safety Assessment of Food Additives

Yoichi Konishi*

1. Introduction

Recently, a variety of foods have entered the marketplace as a result of advances in food production technology. At the same time as this is occurring, health conscious consumers are paying more attention to their dietary habits and to food safety. Since foods contain naturally occurring chemicals as well as chemicals added for special purposes, starting in 2003 food safety began to be assessed by the Japanese Food Safety Commission under the Food Safety Basic Law in order to deal effectively with food supplies for consumers. The safety assessment of environmental chemicals and food additives has depended mainly upon the results of genotoxicity tests and animal experiments, and Japan has not benefited by use of contemporary non-experimental or computational risk assessment methodologies. The Threshold of Toxicological Concern (TTC) is a concept referring to establishment of a human exposure threshold value for groups of chemicals, below which there would be no appreciable risk to human health. This concept proposes that a safety level value can be identified for many chemicals, including those of unknown toxicity, based upon of their chemical structure. The aim of this article is to present the usefulness of TTC for the safety assessment of food additives and to propose of its application in the international harmonization for the safety assessment of food additives.

2. Safety assessment of environmental chemicals

The safety assessment of environmental chemicals has mainly depended upon the results of animal experiments and genotoxicity tests. The National Toxicology Program (NTP) was organized with the support of National Institute of Environmental Health Sciences (NIEHS) and 2-year carcinogenicity bioassays in rats and mice were established in the late 1970s. At almost the same time the Japanese Ministry Welfare and Health recognized the importance of rodent bioassays and a group consisting mainly of pathologists from academia was organized. Test compounds for 2-year carcinogenicity bioassays in rats and mice were selected among compounds that showed positive mutagenicity and were socially important. However, it became clear that as positive or negative separate results between rats and mice were reported that the results of 2-year bioassays and mutagenicity tests were not well correlated. Furthermore, there has been considerable discussion on the necessity of continuing with the bioassay, the utility of alternative methods, international of nomenclature and diagnostic criteria, the selection of historical control data, and molecular mechanisms of tumor development. Therefore, the circumstances concerning the long-term carcinogenicity bioassays are complicated, especially for extrapolation for human risk assessment. Nevertheless, the bioassay is still important but it requires that industry and relevant ministry leaders gain a better understanding of its

* M.D., Ph.D., Professor Emeritus, Nara Medical University
Visiting Professor, Oklahoma Medical Research Foundation

utility and when its use is necessary. The basic concept of the safety assessment of environmental chemicals is shown in Figure 1. When the hazard or risk is identified, the process of risk is characterized; risk evaluation and risk management should be communicate to the consumers.

Figure 1 Basic concept of the safety assessment for environmental chemicals

3. Threshold of Toxicological Concern

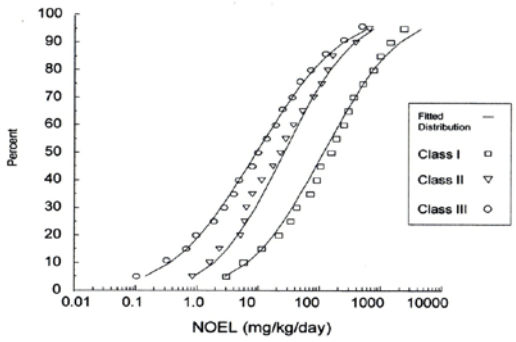
The TTC concept has evolved from a long history of attempts by scientists over the years, in regulatory authorities and elsewhere, to develop generic approaches to the safety assessment of large groups of chemicals or of individual chemicals of unknown toxicity. The proposal for generic TTC was made by grouping according to chemical structure as shown in Table 1. This grouping was based on a reference database which was built up using the result from oral toxicity test in rats and rabbits on 613 chemicals with a wide range of structures and uses. The tests included sub-chronic, chronic, reproductive and developmental toxicity studies. From there, the most conservative no-observed effect level (NOEL) for each chemical was selected, based on the most sensitive species, sex and toxic effect. The 613 NOELs were then plotted in three groups, according of structural class (Figure 2). The driving forces behind these efforts have been:

--- the continuing improvement in analytical capabilities which allows for more and more chemicals to be identified in food in lower and lower concentrations,
--- the widely accepted tenet that exposure to very low amounts of chemicals is usually without harm,
--- the view that the time and attention devoted to a particular chemical should be in proportion to the risk to health from its use,
--- the limited toxicological resources worldwide, both in capacity for toxicity testing and for test evaluation,
--- the desire to minimize the use of animals; and
--- the ability to analyze large sets of existing toxicity data in order to make predictions about the behavior of other structurally-related chemicals.

Table 1 Structural classes for chemicals within the TTC concept

- CLASS Ⅰ = simple structures efficiently metabolized to innocuous products; anticipated low order of oral toxicity.
- CLASS Ⅱ = intermediate structures (less innocuous than substances in Class Ⅰ, but no positive indication of toxic potential).
- CLASS Ⅲ = complex structures; metabolism to reactive products suggestive of potential toxicity.

Figure 2 Correlation of a structural class with NOEL



4. Application of TTC for the safety assessment of food

The TTC has been applied for the safety assessment of the following food components:

flavors, contaminants, and packing materials. It can also be used to identify analytical data needs and to set "analytical evaluation thresholds" above which toxicological assessment may be indicated. However, it was reported that the TTC approach is not appropriate for the safety assessment of minerals, metals, polymers, biologically concentrated substances, proteins, endocrine disruptors, strong carcinogens, substances affect the gastro-intestinal tract, nano-substances, irradiation, essential elements, and for food used for infants up to 6-months of age. With regard to international concerns on the TTC application for the safety assessment of food additives, there is a basic difference between flavoring substance (FS) and other food additives. Assessment of the FS is performed using a different procedure in JECFA, the US, and the EU, while in Japan the assessment of FSs is done in the same way as for other food additives. The EU Food Improvement Agents Package (FIAP) covering FSs, food additives and food enzymes is outlined in Figure 3. The EU food flavoring regulation provides new classifications of the FSs as presented in Table 2. In the FIAP, approved in January 2008, the regulation of FSs is clearly independent from that of food additives. Furthermore, the classification of the FSs, single or complex, has changed. The fundamental concept of the safety assessment of FSs in JECFA is that specific consideration can be made based upon the usage patterns of very low doses and ascertaining the doses which are considered safe for humans, based on the concept of the TTC for the evaluation of FSs. JECFA evaluated so far about 2000 substances and no problems with the use of FSs in terms of the safety for human health have been identified. The decision trees of JECFA, the European Food Safety Authority (EFSA) and Japan are shown in Figures 4-6. In Japan the first step of the assessment is performed based on the results of genotoxicity and a repeated dose experiment in animals while it is the structural class of TTC in JECFA. In EU, the genotoxicity data are evaluated as the first step, but can be skipped if the actual data of the substance possess common metabolic pathways to similar non- genotoxic substances. And then, the requirements for further toxicity data depends on the level of exposure in comparison with respective to the Cramer class threshold. The basic concept of the Japanese flow chart is dependent upon zero-risk of FSs for human health which is essentially speculative rather than based on scientific evidence.

Figure 3 Frame of food improvement agent package in EU

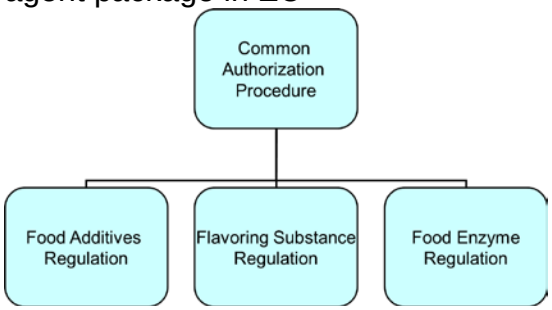


Table 2 Classification of the flavorings based on the definition provided by the new EU regulation

Substances	Past	Present
Single	Natural Flavoring Substance	Natural Flavoring Substance
	Nature Identical Flavoring Substance	Flavoring Substance
	Artificial Flavoring Substance	
Complex	Flavoring Preparation	Flavoring Preparation
	Process Flavoring	Thermal Process Flavoring
	Smoke Flavoring	Smoke Flavoring
	—	Flavor Precursor
	—	Other Flavoring

Figure 4 Flow chart of safety evaluation method applied in Japan for universally-used FSs

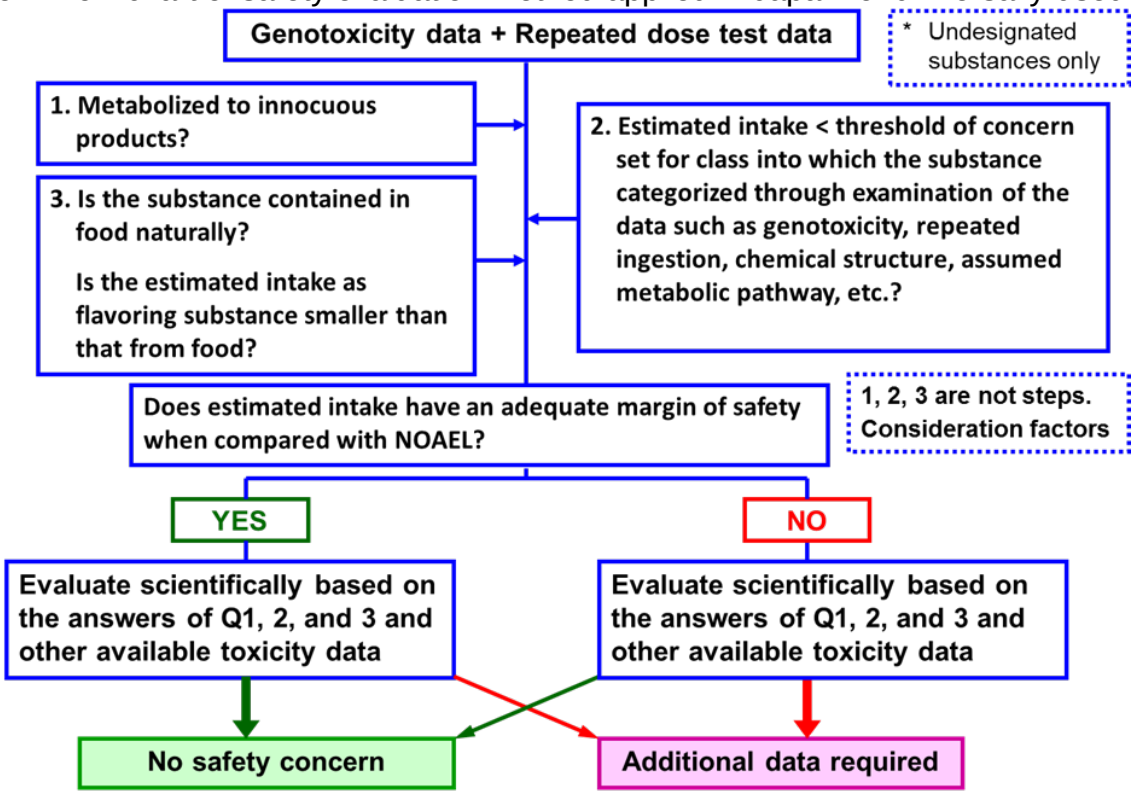


Figure 5 JECFA evaluation procedure for flavoring substances

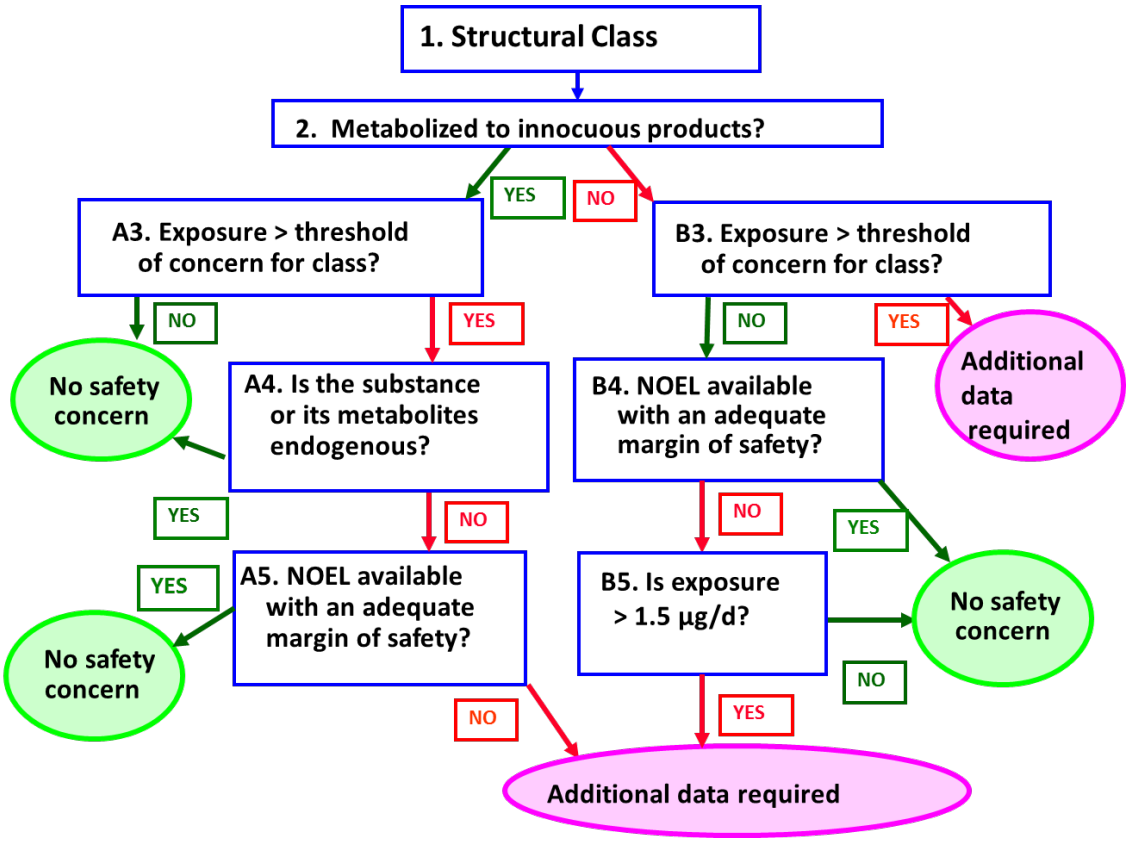
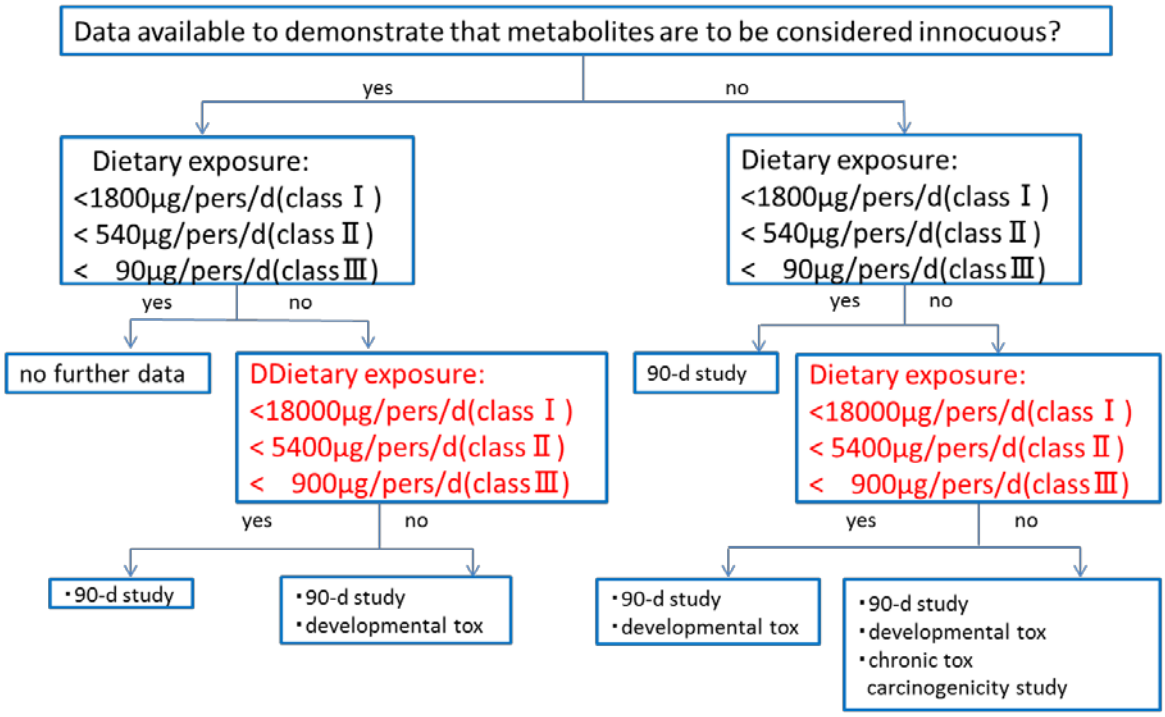


Figure 6 Individual evaluation of the FSs in EFSA



5. Characteristics of flavoring substances (FSs).

The components of general food are shown in Table 3. It is obvious that the amount of FSs used is very low, from ppm to ppb levels. Table 4 shows the estimated amounts of food additives used per year (kg) and the number of substances. There are about 3000 flavoring agents constituting the largest group of food additive agents. The current numbers of FSs used in key worldwide markets is shown in Figure 7. Various flavoring substances are used in Japan, the US, and the EU, including chemically defined FSs as well as natural flavoring complexes. Further, FSs used in different countries have common characteristics such as mostly occurring naturally in food and consisting of mixed-compounds used very low doses, with simple chemical structures and with self-limiting exposures.

Table 3 General components of food

Water	more than 95%
Protein	1～25%
Lipids	1～40%
Carbolydrate	1～80%
Mineral	1～5%
Vitamines	ppm
Flavoring substances	ppm～ppb

6. Possible international harmonization for the safety assessment of FSs.

It is now known that TTC is a useful method to identify safe levels of chemicals based on their structures without the need for animal experiments. Table 5 shows the applicability of TTC proposed by R. Kroes. The relation of structural alerts or grouping chemical structures and daily acceptable doses are described. Recently, EFSA reported how the applicability of TTC schemes can be improved by incorporating physicochemical data and

toxicity data generated by non-testing methods such as Quantitative Structure-Activity Relationships (QSARs) and read-across within related chemical groups. QSAR is a quantitative relationship between a biological activity (e.g. toxicity), which may be categorical or quantitative, and one or more molecular descriptions that are used to predict that activity. A molecular descriptor is a structural or physicochemical property of molecule, or part of molecule, which specifies a particular characteristic of that molecule and is used as an independent variable in QSAR. Read-across consists of qualitative and quantitative analyses. The qualitative read-cross assumption is that more than 2 chemicals which have common structure and with toxicological data among categorized substances that are used are safe. Quantitative read-across is a mathematical analysis using known specific values of more than 2 substances with common chemical structure among categorized substances. These methods indicate the potential of chemoinformatics for exploring relationships between chemical structure and toxicity. Japanese regulatory agencies are paying attention to the methods of safety assessment used in JECFA, the US and the EU, and recently an international symposium to understand and communicate the concept of the TTC, entitled "Usefulness of the TTC for the Safety Assessment" was held in Tokyo. In the panel discussions, agreement was obtained for to use TTC for the safety assessment of FSs and that QSAR is useful as an adjuvant for TTC application. International harmonization for this promising approach should contribute not only to improvement of the smooth business of distribution of FSs internationally but also should address risk communication requirements for human health.

Table 4 Amounts of food additives being used per year (kg) and number of substances

Agents	Amount consumed per year (kg)	Number of agents
Acidifiers	154,042,000	24
Seasonings	110,415,000	54
Non-nutritive sweeteners	87,644,000	7
Dietary supplements	25,276,000	74
Emulsifiers	17,950,000	5
Chewing gum bases	2,600,000	11
Preservatives	2,275,000	18
Anti-molding agents	1,860,000	5
Flavoring agents	1,427,000	about 3,000
Thickening agents or stabilizers	1,114,000	7
Antioxidants	775,200	18
Food colors	685,060	30
Bleaching agents	176,788	5
Color fixatives	93,000	3

Figure 7 The numbers of FSs used in Japan, US and EU

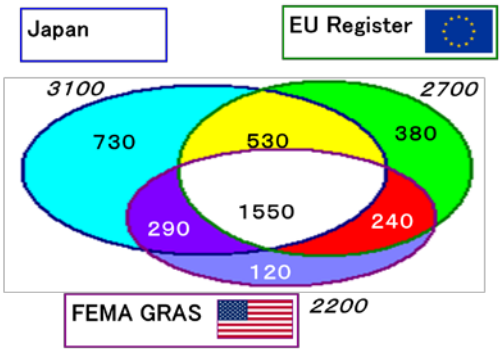


Table 5 Possible International Harmonization for the Safety Assessment of FSs (proposed by Robert Kroes)

How to apply the TTC?

- Stepwise approach on a case by case basis:
  - Specific structural alerts?→ NO TTC
  - All other structural alerts →TTC 0.15μg/person/day
  - Structural alerts excluded → OP ester? →
  - If OP ester → 18 μg/p/day
  - Class Ⅲ chemical? → 90 μg/person/day
  - Class Ⅱ chemical? → 540 μg/person/day
  - Class Ⅰ chemical? →1800μg/person/day

7. Summary

General concept of the safety assessment of food additives and applicability of TTC for that are described. Among food additives, FSs have their own characteristics such as oral low dose exposures, simple chemical structures and self-limiting exposures. Based on their characteristic, FSs are categorically different from other additives and the TTC approach can be used for their safety assessment. Using recently developed in silico methods such as QSAR and read-across, indications for the applicability of TTC will be clear, leading to the international harmonization of FSs.

Note

We deeply regret to learn Dr. Ian C. Munro's death and for his great contribution to the development of TTC.

Acknowledgments

This work was supported by the grant from The Japan Food Chemical Research Foundation and the appreciation to Dr. Shim-mo Hayashi for his cooperation of the collecting information and Mr. Toshinao Baba for manuscript preparation.

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Philanthropic Programs of ILSI Japan CHP

Takashi Togami*

1. ILSI Japan CHP

ILSI Japan CHP (Center for Health Promotion) was established in 2001 to conduct philanthropic programs. ILSI Japan CHP has been contributing health promotion of high risk population through science-evidenced research and investigations on health, nutrition, public health and environments. ILSI Japan CHP develops and implements practical and workable solutions under tripartite partnership of public, private and academia.

ILSI Japan CHP is conducting three projects (Figure 1). These are Project PAN (Physical Activity and Nutrition), Project IDEA (Iron Deficiency Elimination Action) and Project SWAN (Safe Water and Nutrition). Each of the projects is briefed below:

Figure 1 ILSI Japan CHP

2. Project PAN

Physical exercise and good diet practice are most important to promote healthier aging. Project PAN is pursing two different approaches. One is a high risk approach in worksite, LiSM 10 ! (Lifestyle Modification 10 !), which developed an effective health promotion program based on individual counseling. Another is a population approach for the elderly (TAKE 10 !), which developed a comprehensive and sustainable education program to keep the elderly out of being bedridden.

Ministry of Health, Labour and Welfare requests health insurance unions to take measures to prevent metabolic syndrome. LiSM 10 ! was designed to meet the objective and scientifically verified the effectiveness[1-3]. The process is shown in Figure 2. The features of this program is 1) individual objective setting and action plan for physical exercise and diet, 2) self-monitoring and recording 3) periodical counseling emphasizing self– motivated improvement. The counseling can be done either by face-to face discussion or internet. The results of a 6-month intervention program done in Nichirei Food is shown in Figure 3 as an example. The LiSM 10 ! group indicates that significant improvements were observed in 14 parameters while the control group (conventional program) showed improvements in only 7 parameters.

TAKE 10 ! Program recommends that the elderly conduct 10 minutes physical exercise using body weight 2-3 times a day and take 10 different food groups a day. An intervention study was conducted for one year in Nangai community recruiting 1,400 elderly subjects. The study showed that walking speed was maintained and physical exercise habit was established and that the nutrition status was significantly improved by the dietary habit change[4].

* Director, ILSI Japan Center for Health Promotion

Figure 2 The 3rd version in 2006-2007

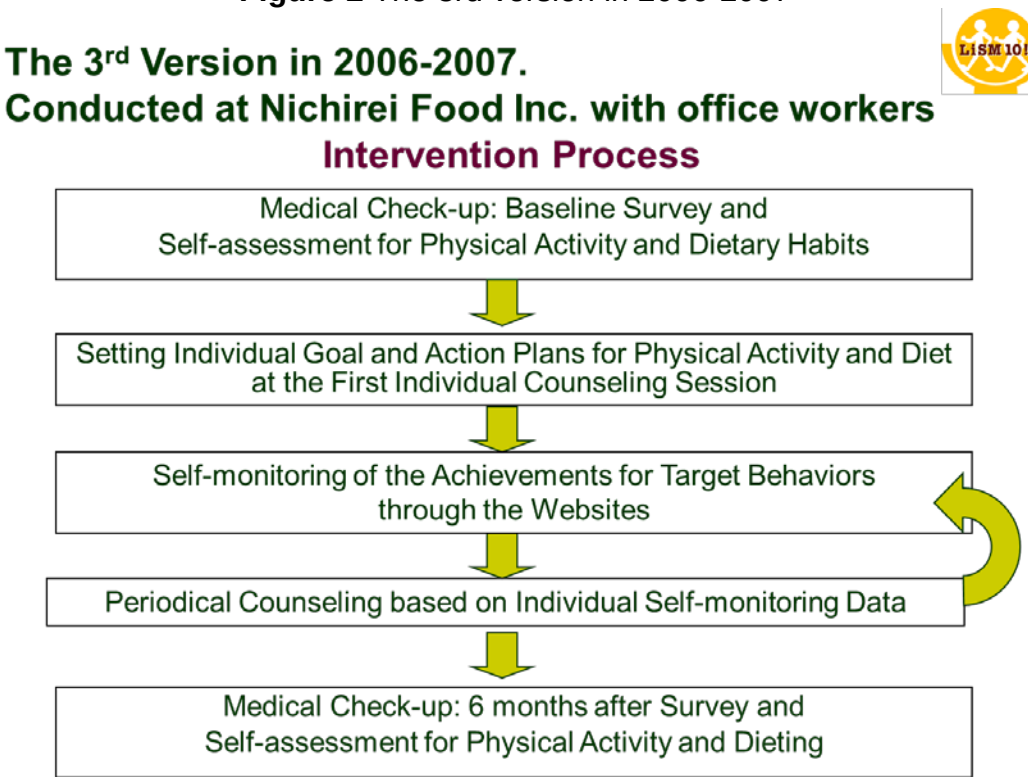


Figure 3 Outcome of the 3rd Intervention study of LiSM10!
(C. Maruyama *et al.*[3])

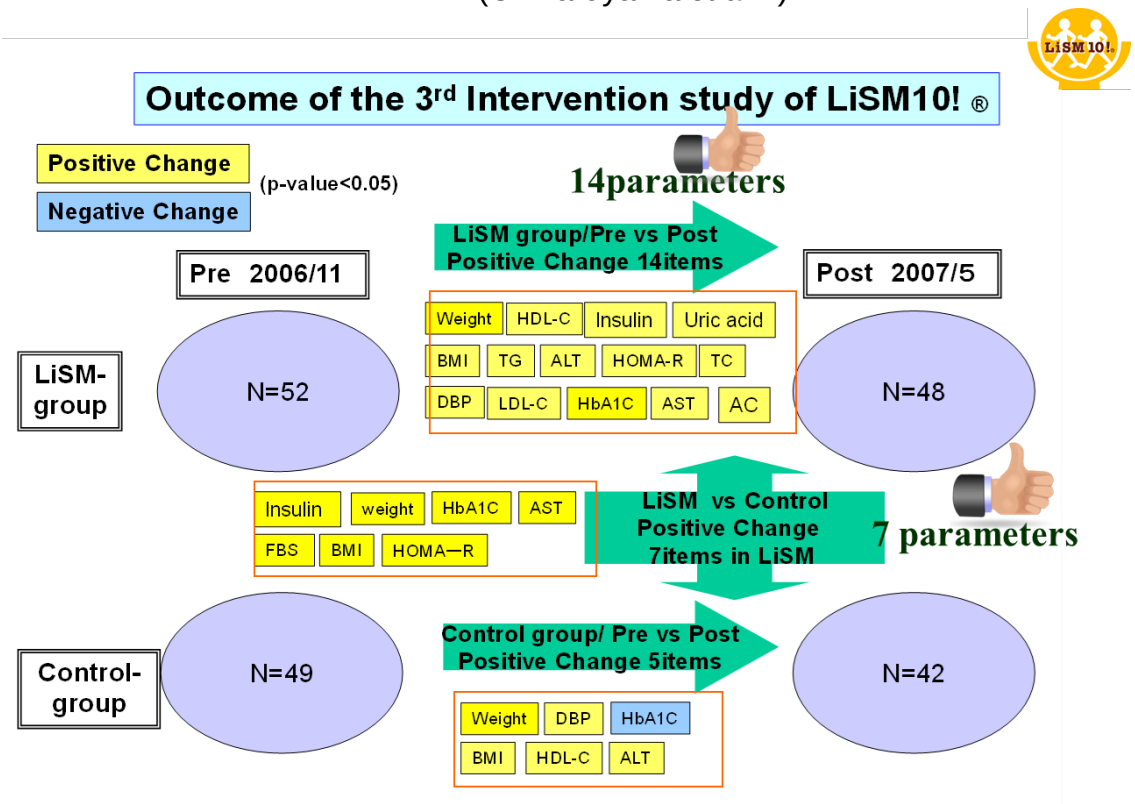


Figure 4 Promotion of TAKE10!

A variety of education and advocacy materials such as manuals, booklet and DVD were developed to facilitate the implementation. As shown in Figure 4, TAKE 10 ! Program has been implemented in various organizations such as provincial governments, the elderly NPOs and the private sectors

3. Project IDEA

The UN ACC/SCN reported that 3.5 billion people suffer from iron deficiency anemia (IDA), and that it has been more difficult to overcome IDA than other micronutrient deficiencies. Project IDEA aims to alleviate IDA in developing countries by fortifying commonly-eaten foods such as condiments and staples based on the dietary patterns unique to each country. This project has continued for more than 10 years. China introduced soy sauce fortified with NaFeEDTA on a nationwide basis and Vietnam commercially introduced fish sauce fortified with NaFeEDTA Cambodia will launch the fortified soy sauce and fish sauce on a nationwide basis soon. In the Philippines and Vietnam a series of research on iron fortification of rice has been pursued. Some of the programs underway are briefed here.

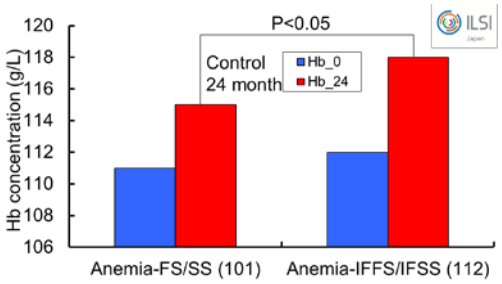
The process of iron fortification is divided into two phases; Research phase and Implementation phase. In Research phase, development work in laboratory is made about fortification level, stability, bioavailability and acceptability of fortificants. Next, development work in the field is conducted in various areas, for examples, efficacy and effectiveness study, production and distribution study, QA system, education and advocacy program, monitoring system, etc. After completion of Research phase, Implementation phase can be started. This phase must start with regulatory compliance coordination in the country and must follow the similar process of new product introduction in industries regarding production, distribution, marketing and public

relations. Two examples of ILSI Japan CHP programs are briefed below.

In Cambodia anemia is a serious public health issue. For example, anemia prevalence among children is as high as 63 %. ILSI Japan CHP initiated collaborative work with a local NGO RACHA (Reproductive and Child Health Alliance) in 2005, aiming introducing iron fortified fish sauce and soy sauce. An efficacy study recruiting school children was conducted for 6 months, resulting in significant improvement in anemia prevalence. Then an effectiveness study or market trial was conducted in Kampot and Siem Reap for 2 years by introducing the fortified condiments. It demonstrated that the fortified fish sauce and soy sauce were effective to improve anemia prevalence (Figure 5). Based on the results, Cambodian government decided to introduce the fortified condiments in 2012.

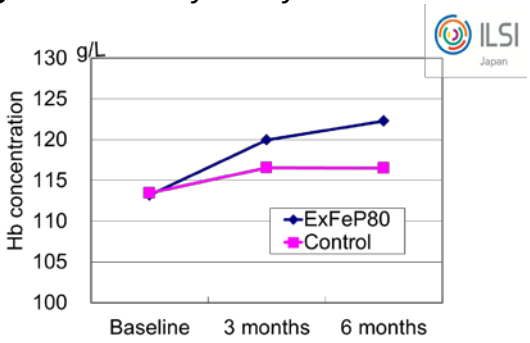
In the Philippines, anemia is still a serious public health issue. ILSI Japan CHP and FNRI (Food and Nutrition Research Institute) have been studying iron fortification of rice as staple food. In laboratory evaluation, it was found that micronized and encapsulated ferric pyrophosphate developed in Japan is most suitable for rice fortification. This fortificant was mixed with rice flour to produce Premix. Fortified rice was prepared to mix Premix with ordinal rice. An efficacy study was conducted recruiting school children, resulting in significant improvement of anemia prevalence (Figure 6). Following up the efficacy study, a market trial was carried out in Orion Municipality in 2008-2009 to evaluate the effectiveness of the fortified rice and its advocacy/education program. As a result it was shown that improvement of anemia was confirmed among school children of 6-9 years old and reproductive female groups and that the education/advocacy program was found effective in the communication. The Philippines government is developing the introduction strategy and plan for the fortified rice on a nationwide basis.

Figure 5 Effectiveness of IFFS/IFSS on anemic women reproductive aged during market trial at SR & KP



Y. Nakanishi *et al.* National workshop, In Cambodia, July 2010.

Figure 6 Efficacy Study of Fortified Rice



4. Project SWAN

WHO reported that 1.1 billion people do not have access to safe drinking water. In many developing countries, the intake of unsafe water and unhygienic environments cause diarrhea and infectious diseases among children. Project SWAN (Safe Water and Nutrition) is conducted by ILSI Japan CHP and NIN Vietnam (National Institute of Nutrition in Vietnam) as JICA Grass-root Technical Cooperation Project. This project aims to establish workable models for sustainable supply of clean water and effective health communication at household level. For this purpose, it was decided that 1) IEC group (Advocacy/Education group) of the project team was responsible for enhancing knowledge of drinking water, food hygiene and nutrition at the household level, 2) Technical group was responsible for optimizing the operation of water treatment facilities (WTF), and then 3) Water Management Union should establish effective management system for sustainable operation. In three sites in the north of Vi-

etnam, Technical group and IEC group worked in 2005-2008 along with the process as shown in Figure 7. As a result, for examples, the diarrhea prevalence was significantly improved (Figure 8), and the performance of ware treatment facilities were also improved substantially in water supply and efficiency as well as water quality (Figure 9). The second phase of SWAN (SWAN 2) started in 2010, which aims to enhance the capability of Vietnamese experts so that they can promote the concept of SWAN on a nationwide basis themselves. For this purpose, Working Team was established at central government level and Supporting Teams at provincial level which provide training to communities so that multi-disciplinary coordination and training through horizontal communication as well as vertical ones can be achieved to enhance the capability of provincial and community experts and to expand the SWAN program. The expansion is underway in 16 sites. SWAN 2 will be completed in 2013.

Figure 7 Process of project

Figure 8 Prevalence of diarrhea among children

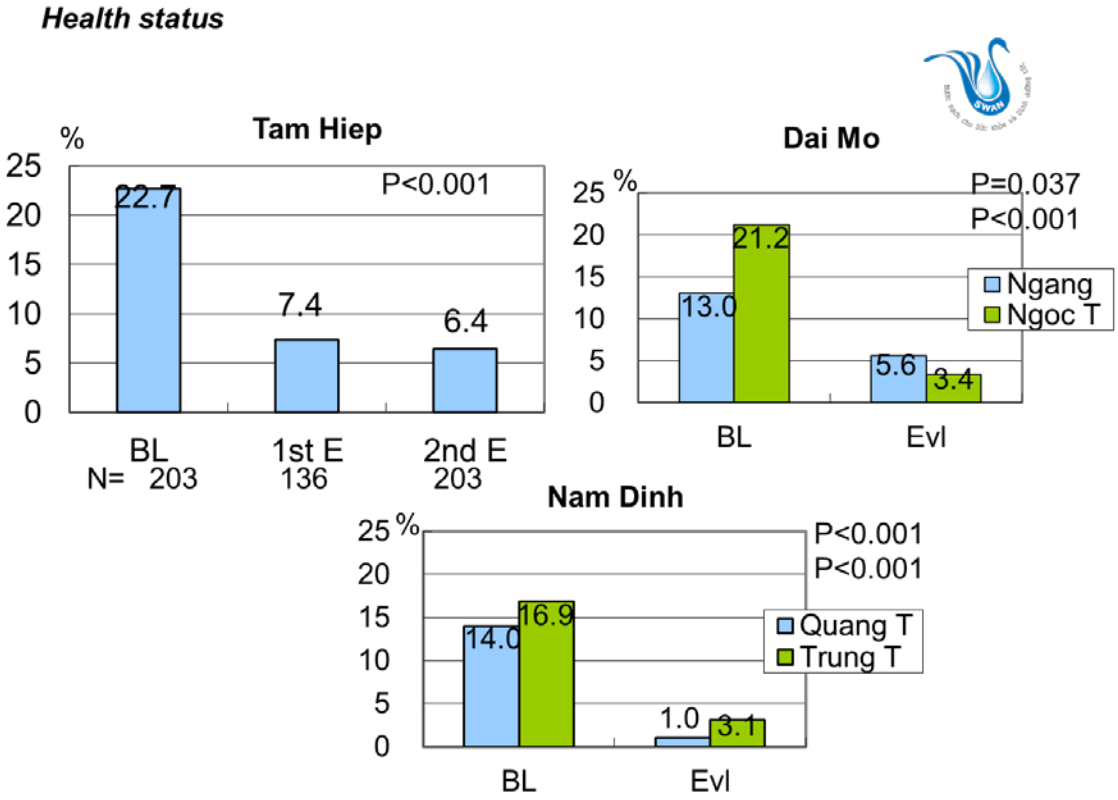


Figure 9 Tam Hiep Commune – Results Improvements in Technical program

Water distribution	BL (Feb '06)	1st evaluation (Jun '07)	2nd evaluation (Aug '08)
No. of total HH in the village	979	993	1,183
No. of HHs received water	638	721	890
Water volume (L/capita/day)	32	52	55
Cost of treated water (VND/m³)	2,500	2,500	2,500
Rate of water loss (%)	54.1	48.8	46.9
Penalty against water theft	None	Under discussion	Applied

5. Closing

Since ILSI Japan CHP was established, 10 years have passed. ILSI Japan CHP has completed substantial programs in three projects. Those programs have been supported by ILSI members, Ministry of Foreign Affairs, JICA (Japan International cooperation Agency), Ministry of Agriculture, Forestry and Fisheries Japan Foundation, The Iijima Foundation for the Promotion of Food Science and Technology in Japan. The programs have also been supported by LISI Research Foundation, UNICEF, and GAIN (Global Alliance for Improved Nutrition). ILSI Japan CHP is very much grateful to those supporters.

ILSI Japan CHP will continue philanthropic programs to benefit high risk people.

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