

# HEALTH AND NUTRITION NEWS

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## Prefatory Note

### National Institute of Health and Nutrition as a brain of dietitians in Japan



Eiji Oshino, MSc. RD.

Director of Nutrition Section, Ishikawa Prefectural Central Hospital

So far, the researches and those outcomes at the National Institute of Health and Nutrition (NIHN) have been regarded as unreachable for the dietitians working in the local provinces.

However, looking at the research outcomes where energy expenditures of general Japanese were actually measured using the doubly labeled water method, and also at the cited references for each nutrient, we can see that more than tens of thousands of articles were reviewed to establish the energy requirements in the recently published “Dietary Reference Intakes (DRIs) for Japanese 2005”. I think, this is exactly the practice of “Human nutrition based on scientific evidence”.

Besides, targeting the nutrition administrative staffs from all over the country, two open seminars titled [Application of “DRIs for Japanese 2005”: Meal planning at the specific facilities for feeding and importance of administrative support] were held in Tokyo and Osaka in May 2005. In addition, under the joint-hosting of NIHN and the Japan Dietetic Association, several training programs were organized in various parts of the country, for which the staffs in charge of DRIs have been working hard even in the weekend too.

In particular, with the application of the DRIs, the registered dietitians are no longer obliged to stick at the theory and recommended values, and that can plan and provide the meals at ones’ own judgments, based on the results of assessment for each patient. This is quite encouraging for the dietitians, indeed. I am happy that the skills of registered dietitians are now highly evaluated, and at the same time, I feel a great responsibility.

We, nutritionists, are often asked about “supplement” at the dietary guidance. In most of the cases, it is actually difficult to answer to those questions promptly using the scientific evidences, while affluent information on health/supplements

have been spread around by the mass media. So, we find that the NIHN website of “Information system on safety and effectiveness for health foods” is useful to search/confirm the answers.

Mass media can provide the information to more than tens of millions of peoples at once. On the other hand, nutritionists can answer to the questions individually only, which sometimes makes me feel rather empty.

Yet, it is also important for the nutritionists to acquire the trusts from the patients by answering questions appropriately. We also utilize the “Starling News” of the NIHN, for the information on the latest researches in the world and also for the literature search for the surveys/researches.

We learnt the goals of Dr. Watanabe, the new Director-General of NIHN, from this newsletter “Health and Nutrition News”, where he says that the NIHN should accumulate not only basic researches on health and nutrition, but also the scientific knowledge on the control of life-style related diseases, and on the Health Frontier Plan. So, we expect that the NIHN will enhance the research/projects, with the emphasis on “Health promotion” and “clinical nutrition”.

I think that these recent research activities of NIHN reflect the spirits of the founder, Dr. Tadasu Saeki, to use the science in the practical activities, and also conversely to investigate the practical activities scientifically, in accordance with the changing social needs in the different times.

The existence of NIHN becomes closer than before, and that it is really indispensable for the dietitians. In particular, the NIHN website and “Health and Nutrition News” are really useful for the dietitians working in the local provinces.

Lastly, I strongly hope that the NIHN will keep supporting the activities of dietitians, based on the scientific evidences.

## Current Research Projects

### Investigation of a new physiological function of nutrients: Vitamin D

Jun Yamauchi

Division of Applied Food Research

We have been working on the researches related to the functions of nutrients. Here, we would like to explain about our studies on a new physiological function in vitamin D, member of the lipid-soluble vitamins.

The precursors of vitamin D go through various processes, and eventually are hydrated in liver and kidney to be an active form of vitamin D (VD). In the target cells, VD passes through the cell membrane and binds to the nuclear VD receptor (VDR). This nuclear VDR-VD complex would regulate gene expression by binding to a specific site of genomic DNA on the target gene promoter. This is a so-called "genomic" effect of VD. It is well acknowledged that there are many studies on physiological function of VD, most of which can be explained by genomic effect. In addition, VD is known by its "non-genomic" effect as well. This means that the effects are, literally, not mediated by gene expression. The main characteristic is that, in the target cells, VD binds to VDR on the cell surface membrane (membrane VDR), and activates some protein kinase, leading to a rapid cellular signal transduction. Therefore, the effects of VD can be observed relatively quickly (**Fig. 1**). Experimentally, the effects would appear in a couple of seconds or minutes when the culture cells are used. On the other hand, even using similar experiments, it takes at least a few hours for the expression of genomic effects. This phenomenon has been recognized since before. However, the mechanism of non-genomic effects of VD is still unclear, as the identity of membrane VDR is unknown.

So, we are trying to identify the membrane VDR using the molecular biological method. Currently,

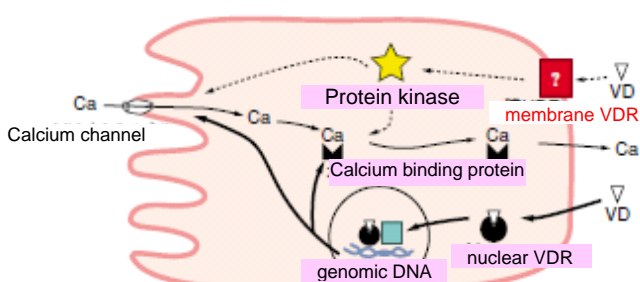


Fig. 1. A model of the physiological functions of vitamin D in calcium absorption. Thick full lines show genomic effects and dotted lines show hypothetical non-genomic effects.

cell strains that activate a kind of protein kinase in response to VD were obtained (**Fig. 2**) (**Ref. 1**). Further analyses will be bringing us to explore the identity of membrane VDR.

Well, for what purpose does membrane VDR-mediated non-genomic actions exist, then? As we mentioned earlier, most of the physiological functions of VD can be explained by genomic effects. Yet, we hypothesize that genomic actions may work together with non-genomic ones. Now, let me take the effects for promoting calcium absorption as an example, as it is the most important physiological functions of VD. Calcium is absorbed in small intestine, and it is said that the absorption can be promoted by genomic effect of VD, as it increases the expression of genes; e.g. calcium channel (for induction of calcium into the cells) and calcium binding protein (that functions for cellular calcium transportation). In fact, however, it is likely that the calcium absorption occurs before gene expression. So, we suppose that non-genomic response would occur just after the addition of VD, followed by genomic effects (**Fig. 1**). Since this is a hypothesis, we have to explore the identity of membrane VDR first, so as to confirm it.

Recently, it has been reported that membrane and nuclear VDR might be the same molecule. The relationship of "genomic" vs. "non-genomic" of VD, therefore, seems still veiled in mystery.

Ref.1) Yamauchi J. The establishment of HeLa cell demonstrating rapid mitogen activated protein kinase phosphorylation in response to 1 $\alpha$ , 25-dihydroxyvitamin D<sub>3</sub> by stable transfection of chick skeletal muscle cDNA library. *Biosci. Biotechnol. Biochem.*, in press

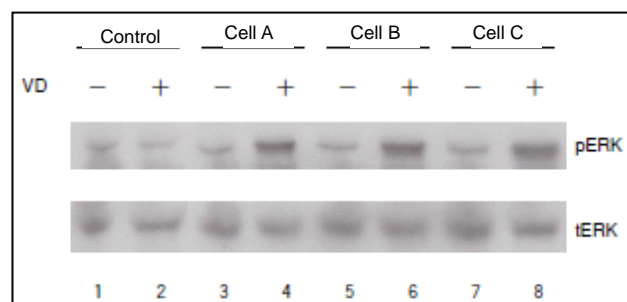


Fig. 2. VD Induced Phosphorylation of ERK in Selected HeLa Cells. The selected HeLa cell lines of A, B, C, and control were treated with or without VD for 1 min. The extracts were run on 4-12 % SDS-polyacrylamide gels. Western blot analysis was carried out using pERK1/2 and tERK1/2 antibodies.

# Thoughts on Health and Nutrition Research

## About “Dietary survey”...

Nobuo Yoshiike

Division of Health and Nutrition Monitoring

Recently, the importance of “nutritional assessment” has been greatly emphasized in various activities for improving the diets and nutritional conditions of the populations. There are diversified target populations and approaches for the “nutritional assessment”. Of which, dietary survey is known to be the most important as a start point of a series of assessments, because it examines and evaluates quantitatively “what and how much an individual (in the personal health service) or population of a community (in public health service) would eat”, Many nutrition experts like the registered dietitians have been conducting “dietary survey” in both practical and academic fields. Most of all, the National Health and Nutrition Survey has a history of about 60 years.

In the field of “Nutritional Epidemiology” which aims to explore the associations between the amount of habitual nutrient intakes and occurrence of diseases, many countries have attempted to develop the self-recording questionnaire applicable in a large-scaled survey (e.g. Food Frequency Questionnaire). Japan has also achieved a significant progress in this research field. On the other hand, for the semi-weighed recording method or recall one, the types and amounts of food items taken on a certain day (mostly on the previous day) should be precisely examined, and thus, various skills are required to the survey teams (e.g. interviews, checking, recoding, data entry and analyses, interpretation etc.). Actually, it is quite difficult for the persons who have not actually implemented the survey to images the types/level of required skills. These skills are never explained enough in the textbooks, and none of them can be acquired in a short term, like the pathognomy in the medical education.

In our Division, we have been working on the development of more precise measurements and improvement of skills of the survey team members for the dietary intake survey (one of the components in the National Health and Nutrition Survey), especially during the past 5-6 years.

Having the cooperation and comments from the survey team members who have been actively involved in the data collection, we repeatedly conducted the pretest of methodologies at the local level. This process helps us to keep the “sense” acquired through practical work, so that we can avoid following an academic theory only.

There still remain quite a lot of things to be done on the dietary survey; research, development of methodologies, skill improvement and education at the registered dietitian training schools. Of which, a particular attention should be paid on the education, as I heard that the education/training are still inadequate in some of the schools for registered dietitian. Here, let me bring an example of medical education again. If one has never seen patients for medical practice, s/he is not supposed to provide the lectures on pathognomy. Likewise, I expect that the research and education on the dietary survey will be greatly developed, if the experienced registered dietitians are actively involved, At the NIHN, we aim to enhance the researches in this field, in collaboration with the active dietitians/nutritionists.



### 6-O-carboxypropyl- $\alpha$ -tocotrienol has an anti-carcinogenic effect against lung adenocarcinoma cells Tomohiro Yano (Division of Applied Food Research)

Vitamin E, as represented by  $\alpha$ -tocopherol, is well known as lipid-soluble antioxidants. It has been reported that the antioxidant effects of Vitamin E could reduce the risk of lifestyle-related diseases. Similarly, the results of past epidemiological studies and animal experiments explored the effects of Vitamin E to reduce the risk of some kinds of cancer. On the other hand, however, it has been also explored that Vitamin E radicals, which are produced when Vitamin E exerted the antioxidant effects, could promote the development of cancer. Similarly, several studies of cell cultures reported that it is not  $\alpha$ -tocopherol itself that has the inhibitory effects against cancer cell proliferation, but some derivatives to block the antioxidant property of  $\alpha$ -tocopherol through ester bond. It is therefore presumed that anticancer activity of Vitamin E is attributed to the structure of non-antioxidant property.

It is known that, generally, tocotrienols possess more potential anticancer properties than tocopherols, possibly due to the difference of the side chain between tocopherols (saturated phytyl chain) and tocotrienols (unsaturated farnesyl chain). Studies have shown that, through this farnesyl chain, tocotrienols could suppress the biosynthesis of cholesterol, inactivate the functions of Ras family (one of the oncogene groups), and finally inhibit the proliferation of cancer cells. Tocotrienols are, however, unstable in the body and are not expected to exert the anticancer activity continually, partly due to their antioxidant property. We therefore synthesized a new stable ether derivative, 6-O-carboxypropyl- $\alpha$ -tocotrienol (T3E) to block the antioxidant property through ether bond, and compared the anti-carcinogenic effect of this derivative with that of  $\alpha$ -tocotrienol, using malignant lung adenocarcinoma cells with a *ras* gene mutation (A549 cells).

In this study, the ether derivative of  $\alpha$ -tocotrienol, T3E, showed cytotoxicity against A549 cells at lower pharmacologic concentrations than  $\alpha$ -tocotrienol or 6-O-carboxypropyl- $\alpha$ -tocopherol (TE), while it had no cytotoxic effect against non-tumorigenic cells under the same treatment conditions. Therefore, we suppose that T3E could be a safe anticancer property as it works on the cancer cells relatively selectively. Our further analyses of T3E cytotoxicity showed that, by inactivation of *ras* family molecules and inhibition of survival-growth signal transduction pathway, T3E would accumulate the cells in the G1-phase of cell-cycle and subsequently induce apoptosis. In addition, the degradation of T3E was not observed even using several enzymes; hence, it was confirmed stable in the body as well. In conclusion, our study succeeded in enhancing the anticancer potential of  $\alpha$ -tocotrienol, by blocking its antioxidant activity through ether bond.

### Induction of cytotoxicity in human lung adenocarcinoma cells by 6-O-carboxypropyl- $\alpha$ -tocotrienol, a redox-silent derivative of $\alpha$ -tocotrienol.

International Journal of Cancer. 2005;115:839-846.

Yano Y<sup>1)</sup>, Satoh H<sup>2)</sup>, Fukumoto K<sup>2)</sup>, Kumadaki I<sup>3)</sup>, Ichikawa T<sup>4)</sup>, Yamada K<sup>2)</sup>, Hagiwara K<sup>2)</sup> and Yano T<sup>2)</sup>

1) Department of Biochemistry, Osaka City University Medical School, Osaka, Japan

2) Department of Food Science Research for Health, National Institute of Health and Nutrition, Tokyo, Japan.

3) Faculty of Pharmaceutical Sciences, Setsunan University, Osaka, Japan

4) School of Nutritional Sciences, Nagoya University of Arts and Sciences, Aichi, Japan

**Abstract:** Tocotrienols are one of the most potent anticancer agents of all natural compounds and the anticancer property may be related to the inactivation of Ras family molecules. The anticancer potential of tocotrienols, however, is weakened due to its short elimination half life *in vivo*. To overcome the disadvantage and reinforce the anticancer activity in tocotrienols, we synthesized a redox-silent analogue of  $\alpha$ -tocotrienol (T3), 6-O-carboxypropyl- $\alpha$ -tocotrienol (T3E). We estimated the possibility of T3E as a new anticancer agent against lung adenocarcinoma showing poor prognosis based on the mutation of *ras* gene. T3E showed cytotoxicity against A549 cells, a human lung adenocarcinoma cell line with a *ras* gene mutation, in a dose-dependent manner (0-40  $\mu$ M), whereas T3 and a redox-silent analogue of  $\alpha$ -tocopherol (T), 6-O-carboxypropyl- $\alpha$ -tocopherol (TE), showed much less cytotoxicity in cells within 40  $\mu$ M. T3E cytotoxicity was based on the accumulation of cells in the G1-phase of the cell-cycle and the subsequent induction of apoptosis. Similar to this event, 24-hr treatment of A549 cells with 40  $\mu$ M T3E caused the inhibition of Ras farnesylation, and a marked decrease in the levels of cyclin D required for G1/S progression in the cell-cycle and Bcl-xL, a key anti-apoptotic molecule. Moreover, the T3E-dependent inhibition of RhoA geranyl-geranylation is an inducing factor for the occurrence of apoptosis in A549 cells. Our results suggest that T3E suppresses Ras and RhoA prenylation, leading to negative growth control against A549 cells. In conclusion, a redox-silent analogue of T3, T3E may be a new candidate as an anticancer agent against lung adenocarcinoma showing poor prognosis based on the mutation of *ras* genes.

## Latest Research

### Abnormalities in the amounts of elements and heme biosynthesis-related substrates in IDA patients. Masao Kondo (Division of Applied Nutrition)

Iron deficiency (ID) is one of the most frequent nutrition disorders among humans. WHO has estimated that 4 to 5 billion people, approximately 80% of the world's population, suffer from ID. Moreover, about 2 billion are anemic attributable mainly to ID. Thus, ID is an extremely common disease not only in developing countries but also in developed countries, particularly among children, reproductive-age women and elderly people. Poor nutrition is the main cause of ID in developing countries, whereas unbalanced diets would contribute to ID in developed countries.

Iron in the form of heme, which is chelated with protoporphyrin, is involved in vital biochemical reactions such as oxygen transport and stock, drug metabolism, energy production, intercellular signaling and elimination of active enzymes. Therefore, the symptoms of ID can appear all over the body such as weariness, irritation, depression, headaches, reduced physical capacity and working efficiency, reduced immunity, paleness, alopecia and brittle nail. Additionally, prolonged ID ultimately suppresses erythropoiesis, leading to iron deficiency anemia (IDA). Due to its significant effects on human health, many studies have been done with respect to the diagnosis, treatment and pathogenic mechanism of ID. Yet, few studies investigated the elements other than iron.

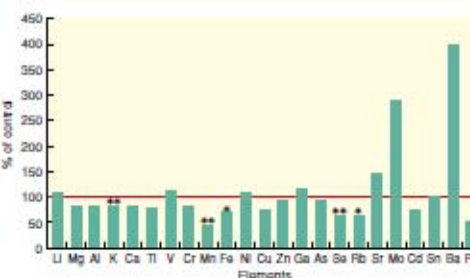
In this study, Zinc-protoporphyrin (ZP) content and the enzymatic activity of both  $\delta$ -aminolevulinic acid dehydratase (ALAD) and porphobilinogen deaminase (PBGD) were significantly higher in IDA subjects (12 women aged 23-38 years were diagnosed as IDA by blood test) than in age-matched healthy controls (Table 1). This result is possibly due to that; since heme can not be produced due to a lack of iron (a substrate of heme synthetase), consequently, these enzyme activities increased under the de-repression mechanism induced by the reduction of heme contents. In particular, ZP is increasingly drawing attention as an index for IDA, and rapid automatic measuring instruments have been developed in other developed countries. Next, the concentrations of trace elements in typical IDA patients were determined by an inductively coupled plasma mass spectrometer (ICP-MS). Figure 1 shows that the amounts of K, Mn, Fe, Se and Rb in IDA subjects were significantly lower than those of healthy ones. On the other hand, the mean Mo and Ba levels were three-fold and six-fold higher in IDA subjects, respectively. So far, no study has reported that the decrease in iron content could affect the concentration of various other elements in IDA patients, hence this study explored an important finding for the prevention and treatment of IDA. For example, since a K deficiency affects the mineral balance in humans and, an Mn and Se deficiency affects the bodies' ability to deal with oxidative stress *in vivo*,

the supplementation of these minerals, in addition to iron, is necessary for the prevention and treatment of IDA. So, we believe, the importance of overall mineral balance in one's body will be emphasized in the future argument on prevention and treatment of ID.

	Control (n=15)	Range	IDA (n=12)	Range
Hematocrit (%)	38.2 ± 3	34.1 ~ 43	30.8 ± 4.98**	20.5 ~ 38
Hemoglobin (g/dl)	13.5 ± 2.54	12.0 ~ 16	9.8 ± 0.28**	9.4 ~ 10.1
Coproporphyrin	1.65 ± 0.56	0.71 ~ 2.93	4.83 ± 2.95**	2.25 ~ 10.9
Free protoporphyrin FP :	13.3 ± 15.1	2.7 ~ 46.5	76.4 ± 149.9	7.9 ~ 519.9
Zn-protoporphyrin: ZP	64.3 ± 23.0	34.1 ~ 116.6	180.1 ± 133.8**	30.4 ~ 519.6
ZP/FP	0.95 ± 5.03	1.65 ~ 18.15	5.46 ± 2.52	0.99 ~ 8.34
Total porphyrins	79.2 ± 35.4	38.8 ~ 154.9	261.3 ± 239.7*	40.6 ~ 796.4
ALAD activity	2.20 ± 0.46	1.56 ~ 2.86	3.83 ± 0.82**	2.54 ~ 4.78
PBGD activity	28.2 ± 6.2	19.9 ~ 38.0	35.0 ± 6.30*	22.4 ~ 45.0

ALAD,  $\delta$ -aminolevulinic acid dehydratase; PBGD, porphobilinogen deaminase; RBC, 赤血球; URO, uroporphyrinogen

**Table 1.** Hematologic data and heme synthesis enzymes and intermediates of patients with IDA



**Fig 1.** Relative levels of the elements in the blood of IDA subjects, compared to the healthy controls

### Iron deficiency anemia: elements and heme biosynthetic system in blood.

**Porphyrins.** 2005;14(2): 99-104

**Kondo M<sup>1)</sup>, Miyamoto H<sup>2)</sup>, Aiba N<sup>1)</sup>, Mori M<sup>3)</sup> and Urata G<sup>1)</sup>.**

1) Laboratory of Nutritional Biochemistry, National Institute of Health and Nutrition, Tokyo, Japan

2) Chiba City Institute of Health and Environment, Chiba, Japan

3) Tokyo Metropolitan Geriatric Hospital, Tokyo, Japan

#### Abstract:

Iron deficiency anemia (IDA) is one of the diseases of worldwide interest. However, almost no research efforts have been made so far on changes in blood trace elements in patients with IDA for the purpose of prevention and treatment of this particular type of anemia, and found out abnormalities in amount of elements in blood of IDA patient's.

Eleven female patients with IDA aged 23~38 and healthy females within the same age range as the patients were employed in this study. Upon obtaining informed consent from these subjects beforehand a heparinized whole blood sample was obtained from each of the subjects and analyzed for blood elements, enzyme activities of heme-synthetic pathway and porphyrins.

In patients with IDA, zinc protoporphyrin,  $\delta$ -aminolevulinic acid dehydratase activity and porphobilinogen deaminase activity were  $180.1 \pm 133.8 \mu\text{g/dIRBC}$ ,  $3.83 \pm 0.82 \mu\text{mol PBG/mlRBC/h}$  and  $35.0 \pm 6.30 \text{ nmol URO/mlRBC/h}$  respectively and were significantly higher than the control value ( $p < 0.01$ ). Concentrations of 23 elements (Li, Al, Ca, Fe, K, Cu, Mg, Ti, V, Cr, Mn, Ni, Zn, Ga, As, Se, Rb, Sr, Mo, Sn, Sb, Ba, Pb) were determined by an inductively coupled plasma mass spectrometer or an inductively coupled plasma atomic emission spectrometer. The average concentrations of Fe, K, Mn, Se and Rb in the patients were significantly lower than the corresponding control values ( $p < 0.01$ ).

These results suggest that the trace elements play a very important role in the prevention and treatment of IDA.

## Latest Research

### An alcohol intake $\geq 300$ g/week is associated with significantly greater annual BP increase in Japanese men. **Katsushi Yoshita (Division of Health and Nutrition Monitoring)**

Hypertension is one of the most important risk factors for cardiovascular diseases, and its prevention and treatment are of international importance. It has been well recognized that hypertension is strongly associated with one's daily lifestyle, including heavy alcohol intake. Most of the past epidemiological studies on the alcohol intake and blood pressure (BP) were either cross-sectional or short-term intervention studies. On the other hand, there were only a few longitudinal studies, most of which examined the long-term effects of alcohol consumption on BP using the onset of hypertension as the endpoint. Yet, little is known about year-long BP increases within the normal range. We, therefore, examined the association of alcohol consumption with BP change over seven consecutive years (1994-2001) in a large adult male population. The study population consisted of male workers at a factory aged 20-59 years. Firstly, detailed baseline data were obtained by a self-reporting questionnaire, which include work-related factors (type of work, shift work, working hours, work intensity, physical/mental stress at work) and lifestyle-related factors (diets, frequency of intake of major food items, alcohol intake, smoking, physical activity levels). BP was measured for all the workers annually at the regular medical examination. Data analyses were performed, taking the changes in systolic and diastolic BP during 7 years as an outcome variable. For which, the recently developed multivariate analysis called "generalized estimating equation (GEE) method" was used to exclude the effects of possible confounders (e.g. work-related or lifestyle-related factors).

The baseline systolic BP (SBP) of drinkers consuming  $\geq 300$ g alcohol per week was 5.21mmHg higher than non-drinkers, after adjustment for baseline age and weight in each year. Annual SBP increase was 0.44mmHg greater in drinkers consuming  $\geq 300$ g/week than in non-drinkers, hence the increase of 3.08mmHg in total during the 7 years' follow-up period. Next, the SBP data were analyzed with adjustment for work-or lifestyle-related factors, in addition to age and weight. Then, the baseline SBP was 4.97mmHg higher in drinkers consuming  $\geq 300$ g/week, with 0.33mmHg greater annual increase, compared to non-drinkers. In addition, even for drinkers consuming  $\geq 200$ g/week, the baseline SBP was significantly higher than non-drinkers. Likewise, the diastolic BP (DBP) data were analyzed with adjustment for age and weight, where the baseline DBP of drinkers consuming  $\geq 300$ g/week was 4.16mmHg higher, with 0.19mmHg greater annual increase, compared to non-drinkers.

In conclusion, this study showed that alcohol intake  $\geq 300$ g/week would significantly influence not only the baseline BP, but also the subsequent BP increase for a long-term in Japanese men. This trend was observed even after the adjustment for various confounders by the multivariate analyses. In Japan, the drinking unit of *gou* is generally used to express the amount of alcohol intake (1 *gou*=180ml of *sake*). So, alcohol intake  $\geq 300$ g/week corresponds to the intake  $\geq 13$  *gou* of *sake* per week. We must note, therefore, that the BP could increase even with fewer amounts than "14-21 *gou*/week", which is generally known to be the amount at risk of hypertension.

It is believed that primary prevention of cardiovascular diseases can be accomplished through interventions conducted widely within the general population, with the objective of achieving a downward shift in the distribution of BP. Our findings of BP increase within the normal range with high alcohol intake would suggest the possibility of suppressing the age-related increase in BP in the general population. We therefore believe that our results provide important evidence for planning the primary prevention measures of hypertension in the future.

### Relationship of alcohol consumption to 7-year blood pressure change in Japanese men

Journal of Hypertension. 2005;23:1485-90

Yoshita K<sup>1)2)</sup>, Miura K<sup>1)</sup>, Morikawa Y<sup>1)</sup>, Ishizaki M<sup>3)</sup>, Kido T<sup>4)</sup>, Naruse Y<sup>5)</sup>, Soyama Y<sup>1)</sup>, Suwazono Y<sup>6)</sup>, Nogawa K<sup>6)</sup> and Nakagawa H<sup>1)</sup>.

1) Department of Epidemiology and Public Health, Kanazawa Medical University, Ishikawa, Japan.

2) Division of Health and Nutrition Monitoring, National Institute of Health and Nutrition, Tokyo, Japan.

3) Department of Social and Environmental Medicine, Kanazawa Medical University, Ishikawa, Japan

4) Department of Community Health Nursing, School of Health Sciences, Kanazawa University, Ishikawa, Japan

5) Department of Community and Gerontology Nursing, Toyama Medical and Pharmaceutical University, Toyama, Japan

6) Department of Occupational and Environmental Medicine, Graduate School of Medicine, Chiba University, Chiba, Japan

**Abstract:** **OBJECTIVE:** To determine the association of alcohol consumption with years-long blood pressure (BP) change, as well as baseline BP, adjusted for potential confounders. **DESIGN:** A prospective cohort study. **SETTING:** A metal-products factory in Toyama, Japan. **PARTICIPANTS:** A total of 3900 men aged 20-59 years. **MAIN OUTCOME MEASURES:** BP was measured annually for 7 years after the baseline examination. The generalized estimating equation method was used to analyze the relationship of alcohol consumption to baseline BP and average annual BP change, adjusting for age, yearly weight, work-related factors, and lifestyle factors, including the frequency of intake of 22 food groups. **RESULTS:** The baseline systolic BP after multivariate adjustment was 3.9 and 5.0 mmHg higher in drinkers consuming 200-299 and  $\geq 300$  g alcohol/week, respectively, than in non-drinkers ( $P < 0.001$ ). The annual increase in systolic BP was 0.44 mmHg greater in drinkers consuming  $\geq 300$  g/week than in non-drinkers after adjustment for age and weight change ( $P < 0.001$ ), where the increase over 7 years was estimated to be 3.08 mmHg greater. Even after being adjusted for the frequency of intake of 22 food groups, drinkers consuming  $\geq 300$  g/week showed a 0.33 mmHg greater annual increase in systolic BP than non-drinkers ( $P = 0.022$ ). Baseline diastolic BP was significantly associated with alcohol consumption, but annual BP change was not. **CONCLUSIONS:** An alcohol intake  $\geq 300$  g/week was associated with significantly greater annual BP increase, and baseline BP was significantly higher in drinkers consuming  $\geq 200$  g/week. It is necessary to limit alcohol intake to less than 200 g/week to prevent hypertension.

### Energy expenditure by intravenous administration of glucagons like peptide-1

Toshimasa Osaka (Division of Human Nutrition)

Various hormones are released from the digestive system after a meal, to facilitate digestion and absorption of foods, as well as to process the absorbed nutrients. Glucagon-like peptide-1 (GLP-1) is one of these hormones, which is secreted from the mucosa of the ileum and colon in response to nutrient ingestion.

GLP-1 stimulates the pancreas to promote glucose-dependent insulin secretion, which works for the uptake of nutrients like glucose by the cells. Thus, promoted insulin secretion means that GLP-1 informs pancreas that "Nutrients are going to circulate through the body now, so please release insulin to process them". In addition, GLP-1 has the function of slowing gastric emptying, by informing stomach that "Since intestine is now busy to digest and absorb the foods currently coming in, please keep the foods in stomach for a while". Likewise, another notable function is the inhibition of food intake, as GLP-1 would work as an endogenous satiety agent who informs the brain that "There are lots of foods in the intestine, so it's not necessary to take foods any more".

It is generally recognized that one of the main meanings of having diets is to take in the energy source in our body. Further more, it is also known that many of the hormones that are released postprandially to work as satiety agents are also associated with energy metabolism. There have been several studies that examined the energy expenditure with intravenous administration of GLP-1 for animals or human. Some human studies, however, provided contradictory results as to whether GLP-1 has a stimulatory or inhibitory action on energy expenditure; intravenous (iv) infusion of GLP-1 increased  $O_2$  consumption, an index of energy expenditure, whereas it decreased meal-induced thermogenesis. We, therefore, examined the effects of GLP-1 on energy expenditure as well as the mechanism of its response, using anesthetized rats. One of the reasons of using the anesthetized rats was that the GLP-1 induced alterations in feeding behavior could secondarily influence energy expenditure. Besides, various surgical or pharmacological manipulations could be applied to the anesthetized rats, so that the physiological functions of GLP-1 can be investigated in details.

In this study, iv administration of GLP-1 apparently increased the energy expenditure of urethane-anesthetized rats. Even with one tenth amount of GLP-1 that causes inhibition of food intake, energy metabolism increased by 20% of the basal metabolic rate and colonic temperature increased by 0.2-0.3°C. Promoted energy expenditure by GLP-1, a satiety agent, means that "Since the energy source comes in now, let's burn it so as not to be obese".

In order to explore the physiological mechanisms underlying this thermogenic response, the effects of GLP-1 were investigated by administering transmission blocker of automatic nervous system or inhibitor of  $\beta$ -adrenergic receptors or by the surgical resection of their organs. Accordingly, we found that the response was mediated by the sympathoadrenal system and  $\beta$ -adrenergic receptors.

The sympathoadrenal system is controlled by the brain. We therefore investigated which part of brain would be involved in the GLP-1-induced energy expenditure by partial ablation or transection of the brain. Decerebration had no effect on the GLP-1-induced thermogenesis, suggesting that the forebrain is not essential for the response. However, cervical spinal transection greatly attenuated the response, suggesting the critical involvement of the lower brainstem.

While "satiety" or "hunger" is determined by the brain, signals that determine such sensations are ingested nutrients in blood or hormones released from the gut. These signals would regulate nutrient digestion/absorption and energy expenditure, as was observed in GLP-1.

### Energy expenditure by intravenous administration of glucagon-like peptide-1 mediated by the lower brainstem and sympathoadrenal system.

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Osaka T<sup>a)</sup>, Endo M<sup>a)b)</sup>, Yamakawa M<sup>a)b)</sup> and Inoue S<sup>a)b)</sup>

a) Division of Human Nutrition, National Institute of Health and Nutrition, Tokyo, Japan

b) Department of Nutrition and Physiology, Faculty of Home Economics, Kyoritsu Women's University, Tokyo, Japan

**Abstract:** Glucagon-like peptide-1 (GLP-1) is released from the gut in response to nutrient ingestion. Intravenous (iv) administration of GLP-1 (50 pmol-20 nmol) elicited dose-dependent increases in the rate of whole-body  $O_2$  consumption ( $VO_2$ ), an index of energy expenditure, and heart rate of urethane-anesthetized rats. The body core (colonic) temperature increased up to 0.3 degrees C without accompanying alteration of tail skin temperature. Intracerebroventricular (icv) administration of GLP-1 induced a slower and smaller increase in  $VO_2$  than the intravenous administration. The injection of glucagon-like peptide-2 (iv or icv) had no effect on  $VO_2$ , body temperatures, or heart rate. Decerebration had no effect on the thermogenic responses induced by the iv administration of GLP-1, suggesting that the forebrain is not essential for these responses. However, cervical spinal transection greatly attenuated the responses, suggesting the critical involvement of the lower brainstem. Adrenalectomy or pretreatment with an autonomic ganglion blocker, hexamethonium, or a  $\beta$ -adrenergic blocker, propranolol, also significantly attenuated the thermogenic response. However, subdiaphragmatic vagotomy or celiac-superior mesenteric ganglionectomy had no effect. Rats made insulin-deficient by pretreatment with streptozotocin also exhibited the normal thermogenic response to GLP-1. These results suggest the involvement of the GLP-1 in postprandial energy expenditure, mediated by the lower brainstem and sympathoadrenal system.