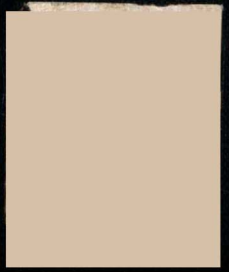
NUTRITION: PROTEINS AND AMINO ACIDS



NUTRITION: PROTEINS AND AMINO ACIDS

Editors
Akira Yoshida, Hiroshi Naito, Yoshiaki Niiyama,
and Takeshi Suzuki

Advisory editors
Norio Shimazono, Itsiro Nakagawa, Kiyoshi Ashida,
and Goro Inoue

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Preface

Nutritional studies on protein and amino acids have recently shown great progress, chiefly due to the improvement of analytical methods and the development of biochemical findings. There remain, nevertheless, many unsolved fundamental as well as practical problems. How to maintain adequate protein nutrition for the world's population at present and in the future given the actual food supply available is a difficult challenge. Nutritional studies should contribute to a settlement of these problems.

This book deals with present knowledge on protein and amino acid nutrition based on their metabolism and physiological functions. Requirements under various conditions are elucidated and safe levels of intake are considered. Malnutrition resulting from protein deficiency and amino acid imbalance, and the methods of preventing and treating such disturbances are discussed. Technological investigations on food and feed preparations are also reported.

The volume was planned and prepared by the Research Committee of Essential Amino Acids (Japan). Originally, an 18-member specialist group was formed in 1956 by the Japan Ministry of Health and Welfare for studies on the utilization of the sources of essential amino acids. When this group was disbanded after three years, the

same members organized the Research Committee of Essential Amino Acids (Japan), which was sponsored by the Japan Essential Amino Acids Association Incorporated. Research continued on protein and amino acid nutrition, and members published the results in assemblies (including symposia) held four times a year. These published papers were printed in the Reports of the Research Committee of Essential Amino Acids (Japan) (ISSN 0387-4141). By the end of fiscal year 1988, 127 assemblies had been held and 120 Nos. of Reports published. Committee members changed during these 30 years and their number has gradually increased, until at present there are 32 members, 35 guest members and 10 associates, which include the foremost active researchers in this field in Japan. This publication commemorates the past 30 years of the activity, and most of the contributors are members or guest members of the Committee.

We express our warm gratitude to the authors and to those who cooperated in other ways in the publication.

March 1989

Norio Shimazono
Goro Inoue

Historical Outline of Studies on Protein and Amino Acid Nutrition in Japan

Norio SHIMAZONO

Professor Emeritus, University of Tokyo

Development of Nutritional Problems in Japan

Traditional food habits in Japan have centered around rice as the staple food with side dishes of vegetable and animal origin. An analysis of the nation's daily per capita food supply made 50 years ago showed that total energy consumption was 2,100 kcal, which included 53 g of protein and 15 g of lipid. The energy ratio of animal to vegetable intake was estimated to be 1 : 20, with the animal portion consisting chiefly of fish and shellfish. During World War II the supply of food throughout the country became very poor, and protein-energy malnutrition appeared in large cities. Immediately after the war the average daily per capita supply was 1,500 kcal, including 36 g of protein and 15 g of lipid, and the ratio of animal to vegetable products was still 1 : 20. Following this period the food supply gradually became better with improvement in the economic status of the country (Fig. 1 A, B).

Before 1940 rice supplied more than 50% of the total energy, but this figure has decreased gradually since 1960, and the percentage is now a little less than 30%. The ratio of animal to vegetable products increased remarkably since 1950, mainly due to the greater

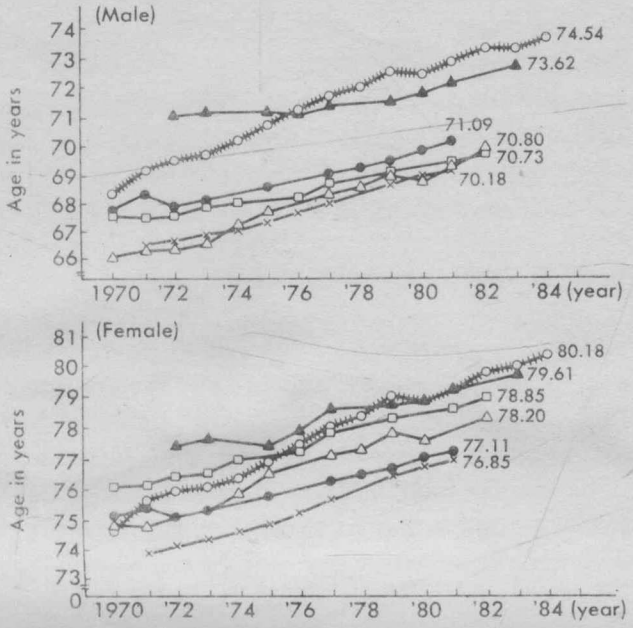

Fig. 7. Changes in average life span: an international comparison. ▲——▲ Sweden; ○-+++-○ Japan; ●——● U.K.; □——□ France; △——△ U.S.A.; ×——× West Germany. (Source: New Social Indicators, National Life Council, 1986, from K. Sonoda: Health and Illness in Changing Japanese Society, 1988)

present, however, especially because of the unbalanced food intake prevailing among some people. Anemia is still predominant in young women and osteoporosis in the aged. Moreover, the development of the biochemical and nutritional studies has elucidated various kinds of inborn errors of metabolism.

At present the lifespan of a Japanese is longer than that of an individual in any other country in the world (Fig. 7). Current nutritional studies are seeking a food pattern most appropriate for a healthy and active life all during this elongated lifespan.

In summary, the changes the people of Japan have experienced and are experiencing in their food habits and the types of food available to them have been extreme during the past nearly 45 years. They have undergone very poor food conditions and, at present, have greater abundance and variety than perhaps at any other time. While this has its advantageous side, it also poses problems of

another nature, as pointed out with regard to health conditions and the obesity now manifesting itself. Yet the development of the studies on nutrition has been valuable and rapid and, together with the rest of the world, there is great desire to determine the most beneficial diet for individuals and to make this information available to them for their enjoyment of a happy life.

Thirty Years of Studies on Protein and Amino Acid Nutrition

The Research Committee of Essential Amino Acids (Japan) was established in 1956 and nutritional studies on amino acids and proteins have been conducted through the close cooperation of the committee members which include many of Japan's foremost nutritionists. The studies have addressed various subjects and the results are difficult to summarize in their entirety, but an outline of the progress made will be attempted here.

1. Protein sources

When the Committee was established in 1956, the supply of protein for Japanese people was insufficient and the search for new sources of high quality protein at a cheaper price was felt to be necessary. *Chlorella senedesmus* and *Torula utilis* were tested not only for animal feed but also for their potential use as human food. Methods of culturing these microorganisms centered on obtaining a uniform quality without contamination, and treatments to remove unpleasant color and taste were evaluated. Digestibility was a problem, and an attempt was made to isolate the protein. Later, single cell protein, which was obtained from microorganisms cultured with hydrocarbon of petroleum, was also studied. Improvement was sought in the preparation of leaf protein concentrate and the seed storage protein, especially for feed.

Methods were investigated to improve the nutritional value of the proteins of rice, wheat, soybean and tropical legumes. Mixing of rice protein and soybean protein was effective. A milk formula composed of several kinds of proteins was reported. Enzymatically modified protein such as methionine-introduced by papain was prepared, and plastein, synthesized by protease, was tested.

2. Utilization of peptides

The nutritional value of peptide was compared with the mixture of the component amino acids. For the preparations of enteral nutrition, protein, peptide and amino acid mixtures were tested. Enzymatically hydrolyzed protein was prepared for elemental diets. Lysino-alanine, which is produced by the alkali treatment of proteins, was examined on its effect.

Some peptides were prepared for special purposes. For instance, peptides with low phenylalanine or low aromatic amino acids were readied for the treatment of phenylketonuria. Peptide sweetenings were developed. Watermelon peptide was reported to be effective for nephritis.

3. Utilization of amino acids

The effect of fortification of cereal protein with lysine and threonine was recognized, and bread fortified with lysine in the school-lunch program improved the physique of the children. To ensure its safety for practical use, toxicological tests were performed on lysine. For soybean protein the effect of fortification with methionine and threonine was examined.

The supplementation of methionine, threonine, branched amino acids, glutamic acid, arginine, histidine and tryptophan was tried for various proteins, and the effects on liver regeneration, muscle protein synthesis, and nitrogen-sparing action were evaluated.

Various amino acids were administered and reported to bring about changes in serum free acids, serum cholesterol, conditions of the circulatory system including atherosclerosis, immunological activity, protein and carbohydrate metabolism, cerebral serotonine, electroencephalogram, enteral microbes and xenobiotics. The utilization of amino acids for enteral and parenteral nutrition, and elemental and low residue diets was studied, and effects on the prognosis of surgical treatment and diseases of liver and kidney ascertained. Taurine and novel natural chloro amino acids were investigated for their influence. Harmful effects of excess administration of certain amino acids and the phenomena of imbalance were observed.

Feeding experiments were performed on hen, chicken, carp,

trout, swine, *etc.* For fish, the dietary use of amino acids coated with casein was devised.

4. Requirements of protein and essential amino acids

As growth is an important factor, the requirements of infants, preschool and school children and students for proteins and amino acids were investigated under various conditions. The needs of adults and the aged were also studied, as was the influence of labor, exercise (sports anemia) and strain. Reports appeared on the relation of protein intake to immunocompetence, atherosclerosis and lifespan.

Studies on nitrogen balance proved useful in estimating human requirements, and estimations were made of obligatory or internal nitrogen loss, especially dermal nitrogen loss. Energy intake was revealed to influence protein requirements. Load tests were made of carbohydrates. Protein storage and turnover rates were studied, and adaptation to low protein intake observed so that the relation of minimal requirement and safe intake level could be discussed.

5. Nutritional value of protein

Methods of estimating the nutritional value of protein were based on animal growth, nitrogen balance, ^{15}N metabolism, body composition, concentrations of blood and urinary constituents, and physiological functions, and subjects discussed concerned chemical score, comparison of protein score (FAO, 1957) and amino acid scores (FAO/WHO 1965, 1973). Reference proteins or the standard patterns of essential amino acids were discussed under consideration of the suggested patterns of amino acid requirements (FAO/WHO/UNU Joint Expert Consultation, 1985). Utilization efficiency was found to depend on protein-energy ratio, the amount of protein intake and intestinal microflora. Adaptation to low protein intake was found to affect the estimates of nutritional value. Some difference was found between amino acid mixtures and peptides of the same composition. Eggs, rice, wheat, soybean, tropical legumes and other foods were evaluated for their nutritional value and utilization efficiency.

6. Assessment and efficacy of protein nutrition

Assessments were made of protein nutrition utilizing nitrogen bal-

ance, nitrogen storage, physique, growth, reproductive activity, mental activity, resistance to stress, xenobiotics, immunological response and other physiological functions. As the body constituents, free amino acids in the blood, liver and other organs and the blood protein (albumin, hemoglobin, *etc.*) were estimated. The effect of training on net working efficiency was found to be an index of protein nutrition.

Nutritional surveys were conducted in tropical and subtropical areas: Tonga, Papua New Guinea, Guatemala, Ghana, Nepal and Indonesia and the findings reported.

Improved protein nutrition was reflected in growth, physique, physical strength, work efficiency, body functions, longevity, resistance to infection, immunological response, xenobiotics, levels of blood cholesterol, insulin and somatomedin, the utilization of iron, secretion of gastric juice, prognosis of surgical operations, stroke prevention, and course of recovery from malnutrition, anemia and hepatic injury.

7. Amino acid metabolism and nutrition

The metabolism of various amino acids—glycine, histidine, glutamic acid, S-containing amino acids, branched amino acids, lysine, tyrosine, tryptophan, *etc.* was studied from a nutritional standpoint, and attention focused on the metabolism of the carbon skeleton of each one.

The selective intake of essential amino acids by rats was studied, as was the regulation of food intake related to amino acid metabolism. Metallic ions such as zinc and copper affected the absorption of amino acids. Amino acids in the intestines were examined. The free amino acid pool in the body was demonstrated.

Metabolic enzymes were found to be induced by ingested amino acids. The relation of niacin to tryptophan and leucine was examined in detail. The metabolic pathway of D-tryptophan and the formation and excretion of taurine were studied.

Amino acids in the blood were determined under various conditions such as adipositas, exercise, vitamin deficiency and hepatic injury. The influence of hormones like growth hormone was also observed, and the transport system of amino acids in the cells

examined. The effects of hormones were determined on amino acid metabolism in cultured hepatic cells. Relation of cholesterol metabolism with amino acid metabolism was observed, and the latter metabolism in muscles was studied by transfusion. Investigations were made of the mechanism of amino acid excretion through the kidney. Amino acid loading tests elucidated the mechanism of formation of fatty liver and the effect of this on hepatic injury.

The relationship between amino acid metabolism and physiological functions in brain was an important subject, and observations were reported on phenylketonuria, hyperornithinemia, cystinuria and hepatic encephalopathy. Studies on the amino acid metabolism in liver under starvation and renal injury were reported. The absorption and adverse effects of lysinoalanine were examined.

8. Protein metabolism and nutrition

Phenomena such as feeding rhythm, selective intake and the regulation of intake were noted in the protein intake of experimental animals. Seasoning, palatability, enteral microflora and dietary fiber influenced protein ingestion. Intestinal absorption was found to occur not only as free amino acids but also as peptides. Dietary protein affected calcium utilization, lipid utilization (infants) and lipid deposits in tissues (eicosanoids, cholesterol, *etc.*).

In experiments on the biosynthesis of protein, stress was placed on ribosomes and nucleoli; the effects of starvation and refeeding were also covered. Biosynthesis regulation was found to occur in accordance with protein intake and under the control of hormones. The activity of the initiation factor was examined under protein deficiency.

The intracellular enzymatic decomposition of protein, especially in cultured hepatocytes, was found to be regulated through amino acids. The turnover of protein metabolism in body tissues (muscles, liver, nerves, *etc.*) was investigated. Its regulation was recognized through insulin and plasma insulin-like growth factor. The effects of vitamin A on the protein metabolism, and of vitamin C on collagen biosynthesis were studied, as well as adaptations occurring when protein intake was low.

Urea was found to be utilized in the body to synthesize protein.

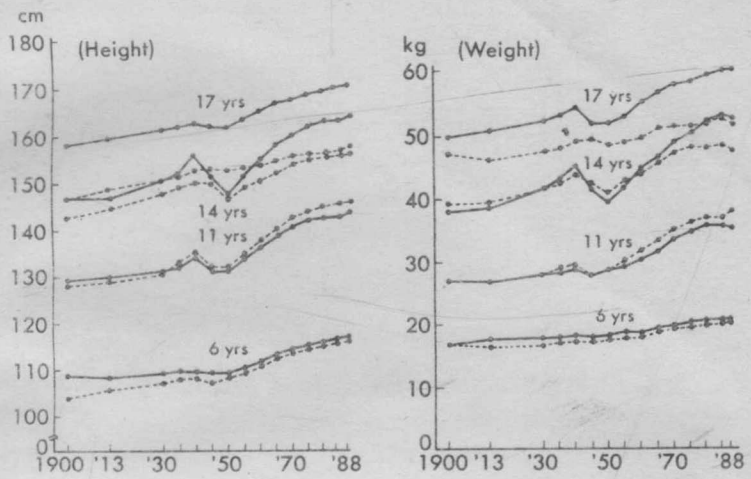

Fig. 4. Trends of physique of schoolchildren and students. ●——● male; ●·····● female. (Source: Japan's Ministry of Education, Science and Culture)

A national nutritional survey has been taken every year since 1950. The results in 1986 showed 2,075 kcal of daily energy intake per capita, 78.9 of protein intake, of which 40.1 g was animal protein, and 56.6 g of lipid intake, of which 27.9 g was animal lipid.

Statistics on the causes of death of Japanese people show that, prior to 1980, the highest three were stroke, malignant neoplasm and heart attack in this order, while the order during 1980–1984 was malignant neoplasm, stroke and heart attack; since 1985 the order has been malignant neoplasm, heart attack and stroke. Strokes are thus decreasing while heart attacks are becoming more numerous (Fig. 5). Stomach carcinoma and liver carcinoma were predominant among the malignant neoplasms, while the incidences of intestinal and mammary carcinoma had long been lower than Western countries. In recent years this pattern has shown a change, becoming more similar to that in Western countries, with the incidence of intestinal and mammary carcinomas rising and that of stomach carcinoma on the decline (Fig. 6).

These changes in causes of death are thought to be largely attributable to changes in eating habits. The incidence of nutritional diseases has also changed markedly. Directly following the war there

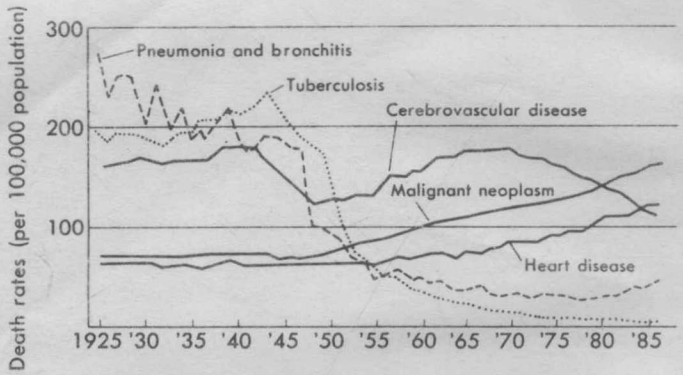

Fig. 5. Trends of death rates by leading causes of death. (Source: Japan's Ministry of Health and Welfare)

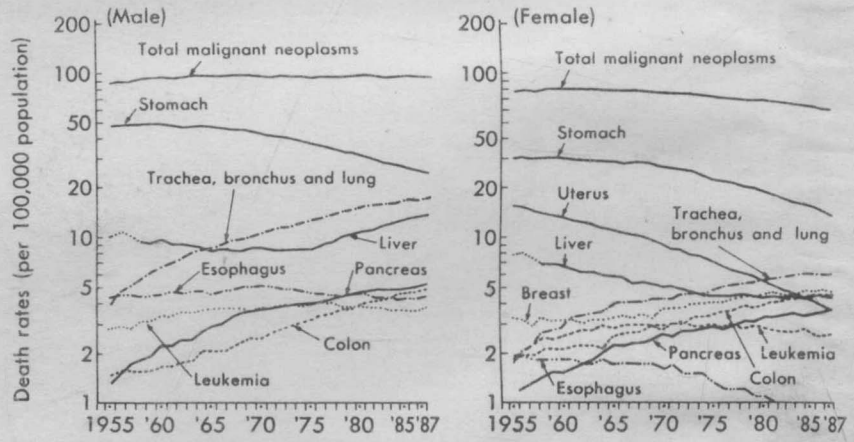

Fig. 6. Trends of death rates from malignant neoplasm by sex and site. (Source: Japan's Ministry of Health and Welfare)

were many sufferers of protein-energy malnutrition and beriberi, but there are now almost none of these, rather, obesity has become prevalent. Sufferers of diabetes mellitus and gout are now more numerous, and nutritional problems have shifted from those caused by deficiency to those due to excess, as well as a great swing in the balance of nutrients.

Marginal deficiency conditions should not be overlooked even at

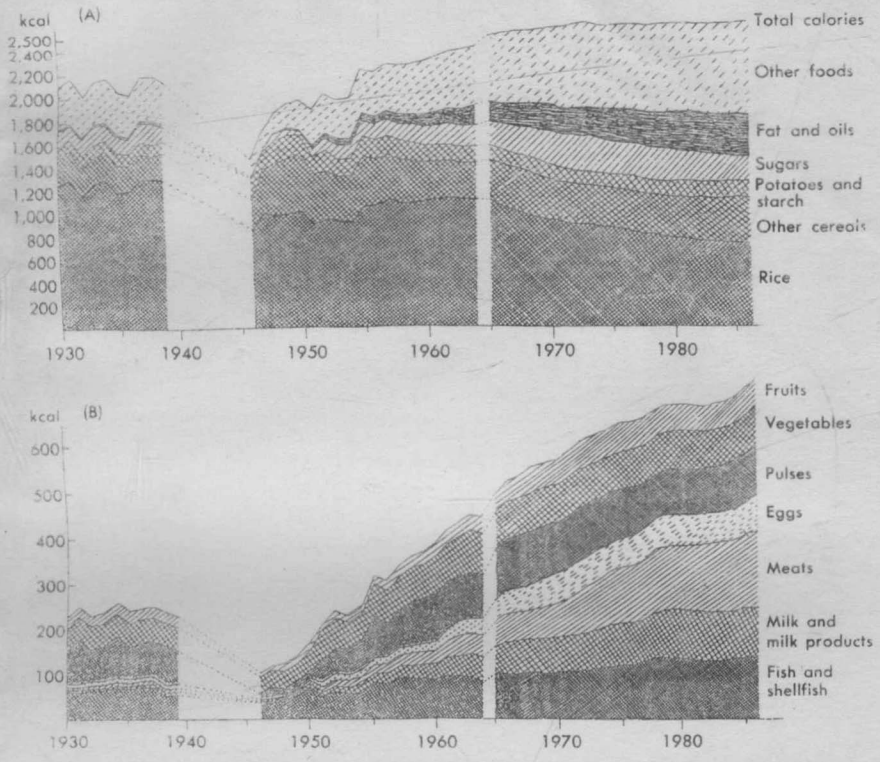

Fig. 1. Trends of net food supplies per capita per day in calories. A: total foods. B: details of "other foods" in (A). (Source: Japan's Ministry of Agriculture, Forestry & Fisheries)

availability of meat, milk and milk products, and eggs. Thus the ratio of protein of animal origin to that of vegetable origin rose to 1 : 1 (Fig. 2). The supply of lipid has also increased notably: 80.5 g at present as compared to the 15 g before 1950. However, the ratio of lipid from vegetables, fish and shellfish to total lipid is still far higher than in Western countries (Fig. 3).

Such changes in food supply are thought to be a main cause of the improved physique of the country's people. The height and weight of schoolchildren and secondary school students (from 6 to 17 years of age) decreased greatly directly after the war, but a gradual recovery took place and the increase is still continuing; current statistics are markedly higher than those prior to 1940 (Fig. 4). A

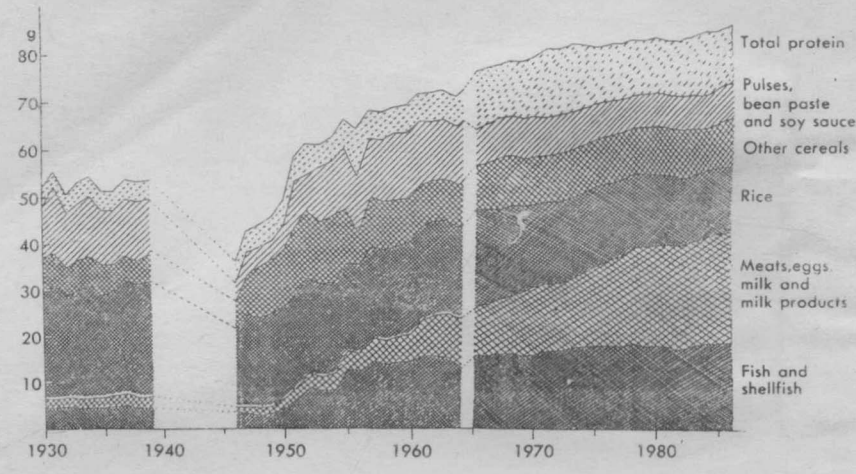

Fig. 2. Trends of net protein supplies per capita per day. (Source: Japan's Ministry of Agriculture, Forestry & Fisheries)

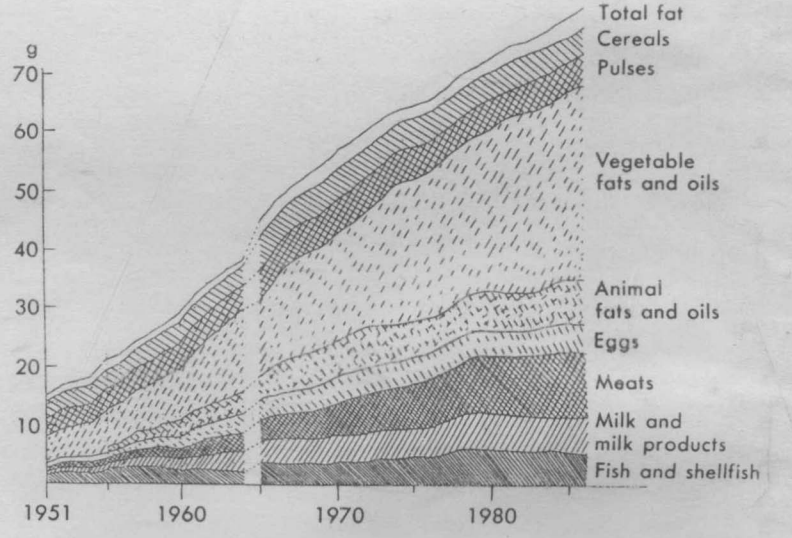

Fig. 3. Trends of net lipid supplies per capita per day. (Source: Japan's Ministry of Agriculture, Forestry & Fisheries)

statistical analysis has shown the correlation of the improvement in physique to the increased intake of nutrients, especially animal protein, fat, vitamin A and riboflavin.

The existence of a peptide pool in muscles was proved, and peptide was recognized to be excreted in urine.

The protein metabolism was examined under various conditions such as vegetarianism, exercise, starvation, pregnancy, infancy, in the aged and in pathological situations (diabetes, hepatic injury, *etc.*), and its effects on immunological response and xenobiotics. It was learned that a middle molecular peptide was responsible for hepatic coma.

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