Reviewers' comments and our responses

The result of evaluations of three reviewers and responses of program leader to each reviewer's comments is as follows;

1) Action class assessment for the research program

[A/B]

2) Comments and suggestions for the research program

The rationale for Food Function research is clear and convincing: prevalence of metabolic syndrome, allergic disorders, asthma is increasing in Japanese adults. Moreover the Japanese populations is superaging, therefore research on quality of life is very relevant. The resulting NARO program 310 has a logical structure namely: Overall, the NARO Research Program 310 in a preeminent health related program with national and international significance. It is necessary to consider the following comments to ensure the achievement of the final goal of this project.

A good description of the concept of functional ingredients and functionality is missing. This is important in order to motivate the choice of the functional components in the separate projects and to keep focus in the research. There are too many individual studies within the four projects. a) Prioritizing of 2 to 3 major components and integration of the four projects would be desirable. To achieve commitment, it would be good to intensively discuss the objectives within and between the research teams. b) Greatly recommend to keep good communication and cooperation with medical school or hospital for the coming researches. This will be very highly valuable for this total program. c) There would be merit in have a facility where short-term controlled feeding investigations could be conducted in areas of eating behaviors and the biological response to bioactive food components. It may be considered to establish a facility for controlled human intervention studies (20-30 participants) in order not to be totally dependent of medical schools for clinical studies.

d) Since limitations in financial support were evident during the review, there is a need to focus and prioritize the research that is being undertaken and ensure as much collaboration occurs within and across each research project. e) Multiple functions of food is the world trend, hope to reach this goal by the team work.

(Response to the underlined comments)

a) We will move forward with the prioritization and the concentration on priority areas of research objects and the strengthening of cooperation among the research projects (310a-310d).

b) We are trying to find scientific evidence in collaboration with medical field (medical school or hospital) in NARO's Research Project on Development of Agricultural Products and Food with Health-promoting benefits. We continue our efforts to carry out the intervention study or the prospective cohort study in collaboration with medical field or Ministry of Health, Labour and Welfare affiliated National Institute of Health and Nutrition (NIHN).

c) It's desirable to hold our own facilities to carry out human trials, but it's hard to get budget for the building expense or the expense of maintenance of the facilities. So, to achieve maximum effect on a limited budget, we will promote and implement joint research with the research institution has the facilities such as NIHN.

d) We will review a collaborative relationship between each research projects or individual subjects, and make further efforts to drum up competitive funds.

e) We recognize the importance of studying on multifunction of foods, so, the studies of functionality using whole food or agricultural products should be located as main research object. Moreover, we will also conduct research of multiple food functionality (such as combination of foods and the development of health functional recipe).

3) Comments and suggestions for the 4 research projects

(1) Analytical and Evaluation Methods for Food Functionality (RP310a)

This Project aims to develop methods for analysis of functional components and the evaluation of their functionalities of agricultural products and foods. This plan is being undertaken by an exceptional strong analytical group. There is unquestionably a need to define the multitude of components that occur in local agriculture products and to evaluate their overall functionality. Thus, the overall goal has major significance. However, this is also a massive undertaking and thus the scientists involved with this project must prioritize which components and functionality are to be pursued. It is also critical this be done in concert with others pursing this area of investigation. a) It is also critical that this team examine the component-component interactions that may occur among food components rather than focusing on single isolated components.

b) The development of a food functionality database is interesting but ambitious. Important to know is where the database is used for. Also linking to other (inter) national databases is

important. [c] The example Kurodamaru describes what the best location is to grow the cultivar. This is interesting but different from the initial objective. Moreover, it focuses on high concentration of anthocyanin, while there are other important components in this soybean cultivar, e.g., genistein. [d] It needs to focus on critical topics that can be achieved in a reasonable timeframe and have the greatest impact of understanding the health benefits of foods consumed by Japanese. [e] Biological system or methodology used for evaluating the antioxidation is greatly recommended. The team is also encouraged to consider other biological properties beyond the antioxidant potential of selected foods/components, such as their impact on cellular growth, apoptosis, differentiation, immunocompetence, etc. that are key factors in disease risk.

(Response to the underlined comments)

[a] We recognize that it is very important to analyze the complex interaction that may occur among food components. We will first start to develop the evaluation methods in order to clarify the interaction of food components in the next medium-term research plan.

[b] We will promote the development of the new database while always conscious user. Especially, we have a plan to perform development of functional agricultural products database in NARO's Research Project on Development of Agricultural Products and Food with Health-promoting benefits. In the project, this database will be connected with the other related domestic databases. This database will increase the volume of data and be connected with the international database in the next medium-term research plan.

[c] The data of "Kurodamaru" is one example for the explanation of the database, and we have a plan to increase contents like a genistein, which also is very important food component in future.

[d] We have a plan to elucidate the effects of some Japanese normal foods on long life of Japanese.

[e] It is necessary to do analysis not only the antioxidant activity of some foods but also relationship between intake of foods and risk of diseases in an epidemiological study. Now, we are starting the trial of the study on the reconstruction support project related with Japanese earthquake 3.11, and also starting to analyze antioxidant "*in vivo*" maker as the antioxidant for research of interventional trial in intake of polyphenols on the functional food development project. We have a plan to analyze a relationship between antioxidant capacity of some foods

and biological reaction in near future.

(2) Metabolic Regulatory Functions (RP310b)

The overall objective of this Project are to elucidate the metabolic regulatory functions of agricultural products which prevent lifestyle-related diseases from NARO's genetic and related resources, and develop food rich in components with metabolic regulatory functions in an exciting area but is also a massive undertaking.

a) The objective of this RP310b to elucidate metabolic regulatory functions is clear, however, the projects described are very different in topic. b) It was not always clear during the review how the multiple projects fit together since each seems a unique approach. The integration of projects into a more manageable thrust seems appropriate.

The effective use of omic technologies by this team is readily apparent. c) The group may wish to explore the use of metabolomics as a quick screening tool for evaluating the functional significance of the foods that are being examined. While this technology is not without issues, especially those related to amounts of foods/components provided and temporal relationships, it may provide some unique insights especially when coupled with transcriptomic information.

d) This project was mainly focused on the metabolic regulatory functions of agricultural products, but most studies were about compounds. These animal models and DNA microarray are available to screen the highly potential agricultural products. e) For further human clinical trial, the potential agricultural product should be very clear and well focused. In addition, end point and the clinical design should be cooperated with medical staffs with expertise of human clinical.

(Response to the underlined comments)

a) In the next medium-term research plan, we will narrow down the items and health functions, and then we will set research issues fit together.

b) This project is conducted in the National Food Research Institute, the Institute of Fruit Tree Science and 4 regional research centers. We have chosen the optimal approach and studied on the important agricultural product of each region cooperatively. Although we are studying in close coordination, the relationship among the topics might not be always clear. As mentioned above, we will narrow down the items and health functions, and then we will set research issues fit together in the next medium-term research plan.

c) We will further undertake research on nutrigenomics including metabolomics, transcriptomics and other omics information in cooperation with the forward-looking

universities and the NARO projects on analysis.

d) In our project, we elucidated not only the metabolic regulatory functions of compounds but also the functionality of the processed food freeze-dried tofu using a DNA microarray analysis. In the next medium-term research plan, we will develop the evaluation method to assess the nutrition and health function using animal models and DNA microarrays, and then we will elucidate the functions of agricultural products.

e) We have selected the potential agricultural products and started the human clinical trials in the NARO functional food project under the cooperation with medical schools and hospitals. In the remainder of the third and the next medium-term research plan, we will strengthen cooperation with medical field, make precise protocols and conduct the clinical trials.

(3) Effective Utilization of Homeostatic Function (RP310c)

The overall objective of this Project is to evaluate and elucidate homeostatic functions of agricultural food products as an approach to increase healthy life expectancy in older aged Japanese and thereby reduce health care cost. Undeniably this is a laudable goal with critical significance to society.

The development of a novel screening method for Natural Killer Cell activation demonstrates the creativity of this group. a) The effective concentration of such investigations always needs to be considered in relation to what might in humans under physiological relevant eating conditions. This becomes particularly evident in the citric flavones studies where the highest concentrations occur in parts of fruits that are not typically consumed. The allergenic studies that are being pursued are also creative and thought provoking. b) The nose blowing studies must be viewed with caution since individual variation would be expected. Other models likely need to be pursued to adequately evaluate the uniqueness/physiological significance of this tea. c) Maybe a pig model instead of a mice model is more appropriate to study immunocompetence. If possible look at multiple cells that are important in immunocompetence. Also the microbiome is of relevance in immuun response. d) For anti-aging, please setup a clear target or function to restore or reach better function.

(Response to the underlined comments)

a) Concerning the citric flavones with NK cells activation activity, we will develop the processing/cooking methods of citrus peels to keep their activity and elucidate their effects on human health in view of innate immunity.

[b] Concerning the rhinitis model, we will develop unbiased evaluation methods other than the nose blowing studies. Provocation test is a challenge to be addressed in addition to natural exposure. Other than rhinitis, we should evaluate the effect of green teas using models of atopic dermatitis and urticaria. We also elucidate the physiological effect of tea through its anti-inflammation effect.

[c] It may be difficult for our situation to use pig as a model animal to study immune reaction. Instead, we will study various kinds of immune cells for evaluation of functional components and also translate these finding into human studies We think that human microbiome is an important target in the next medium-term research plan.

[d] Skin senescence is one of the targets to study anti-aging. We will evaluate the effect of intake of an agricultural product on physiological oxidation state, which is involved in aging, by nutritional and epidemiologic analysis and intervention study.

(4) Flavor and Texture Evaluation (RP310d)

This project is aimed at developing and improving of technologies for evaluating flavor, texture and other sensory properties of agricultural products and foods and the use of communication technologies to share the new information obtained. The major thrust in improving methodologies, the application of these methods for selected foods and the communication of this information are all impressive undertakings by this research team.

The sweet taste receptor is the study target to better understand the intensity of taste. [a] Maybe of interest to consider SNPs (genetic susceptibility to taste) to select a homogenous and responding study population. Not clear is in what way the findings form the transfected cultured cells contribute to food design. [b] It should be noted that odor is also an important factor and should not be avoided. [c] Practical labeling and information to consumers is not properly described. What exactly will be communicated about the results? How will this be translated to the consumer. [d] The well established models in this project should be connected with the other projects of this program, this could make the successful products available (e.g. Benifuuki green tea). [e] For flavor, texture, it would be preferred to work more with food industry, because it is expected that much expertise is present on sensory attributes of foods.

(Response to the underlined comments)

[a] We are going to plan studying of application of SNPs as one of our important themes. Practical food design using the basic results that effective combination of sweet substances to

reduce sugar content in foods obtained from the cultured cells is also very important area. We will study it in the next medium-term research plan.

b) We are studying odor of roasted tea now, and wider studies on food odors related to palatability will be conducted in the next medium-term research plan.

c) As it has been originally planned for the late half of the 3rd mid-term, we have just started to test effective ways of informing food qualities gotten in the first half of the mid-term to consumers. We have not the results yet.

d) Flavor and texture characteristics are important function on daily food in Japan as explained in the review by Dr. Ohtani. This project on palatable properties and the other projects on physiological functions are integrated and we will elucidate “Nutrition and health function in food” in the 4th mid-term. Our proposed models to evaluate flavor or texture properties will be considered in the coming project.

e) We presently conduct collaborative studies with about 30 partners and are going to do more with the food industry.