Impact Objectives

- Explore novel ways to prevent unwellness and disease caused by lifestyle habits, including food additives, daily stress, eating habits and environmental factors
- Study chronic oxidative stress and how it can be controlled or avoided with certain lifestyle changes

A concept for continued health

*In interrelated studies, **Professor Yukiko Minamiyama** is exploring the full depth of the exposome concept in order to advance understanding and facilitate changes that will promote health and wellbeing*

Could you provide insight into your key research interests?

The majority of biological signalling is done by redox signals, phosphorylation signals and gas mediators. I am interested in the production of free radicals *in vivo* and their modification of various proteins and nucleic acids, etc. These free radicals are essential to the organism for sterilisation and other purposes, but when produced in excess, they attack normal cells. Currently, ageing is also recognised as a disease, and the 'free radical theory' is gaining ground. I am seeking ways to prevent unwellness and disease caused by lifestyle habits. This includes not only food additives, but also daily stress, eating habits, environmental factors such as fine particulate matter (PM2.5), radiation and ultraviolet rays.

You are looking at the actions of various sweeteners on rat sperm. Can you explain more about this?

Human sperm counts have declined by about half in the last 50 years. In addition, infertility treatments are increasing at an accelerating rate, and we believe that male infertility may be caused by dysfunction due to excessive reactive oxygen species (ROS) production from sperm mitochondria caused by exposomes (various chemical substances).

You are also studying food additive-induced oxidative stress in rat male reproductive organs and the hippocampus. What does this involve?

The study evaluates, among other things, the amounts of oxidation-modified proteins in the testes and hippocampus of rats treated with up to eight weeks at 1/10,000 of the non-toxic dose of a fungicide applied to imported citrus fruits. The sites of oxidative modification are spermatocytes, which are the source of sperm production in the testes, and the dentate gyrus, which controls short-term memory in the hippocampus, suggesting that they are associated with infertility and dementia.

Can you discuss your experimentation methods and some of the findings you have unearthed?

Since it is not possible to experiment on humans, we mainly use rats in our experiments. Under the Food Sanitation Law, food additives are always tested on animals in the same manner as pharmaceuticals, and non-toxic amounts are calculated. Generally, 1/100 of the non-toxic amount is used as the amount that will not cause harm even if ingested by humans over a lifetime. We have reported that ROS leaks from rat sperm after only two weeks of administration, even at 1/10,000 of the non-toxic dose. However, food additives are ingested in a complex manner, and their effects are completely unknown. Therefore, we are currently conducting molecular biological studies on the *in vivo* effects of a mixture of various food additives at 1/10,000 of the non-toxic dose, focusing on oxidative stress using rats.

What is the expected impact of your work?

Our research shows that food additives undoubtedly induce oxidative stress *in vivo*. We are trying to prove that as long as food additive intake is unavoidable in the diet, it can be avoided by simultaneously consuming antioxidants that would reduce the oxidative stress caused by it. It has been reported that food additives, including artificial sweeteners, are risk factors for changes in the diversity of intestinal flora and various diseases. Therefore, we believe that avoiding these changes will help maintain the health of humankind.

Exposing the secrets of the exposome

*At the **Graduate School of Life and Environmental Sciences, Kyoto Prefectural University** research in the burgeoning area of exposomes could reap significant benefits for human health*

On a daily basis, humans encounter various environmental exposures, both externally and internally. From the molecules that are produced naturally in the human body as a by-product of metabolism or by exposure to toxins in the environment; so-called free radicals to fine particulate matter, exposures are all around (and inside) us and seemingly unavoidable. But, over a lifetime, cumulative exposure to these factors can be damaging. A relatively new concept, termed the exposome, refers to the chemicals that humans ingest during their lifetime. It can be defined as the totality of exposures and their effect on human health.

Professor Yukiko Minamiyama of the Food Hygiene and Environmental Health Division of Applied Life Science at the Graduate School of Life and Environmental Sciences, Kyoto Prefectural University posits that lifestyle changes could reduce exposure to these chemicals and thereby maintain human health. She is also interested in chronic oxidative stress and how it can be controlled or avoided with certain lifestyle changes.

Various studies are being conducted by Minamiyama's team with a view to delving deeper into the concept of the exposome and chronic oxidative stress in order to facilitate informed changes that could prove beneficial to humankind. This work relies on important collaborations with experts in the topics studied. 'I am able to engage in my research only with the cooperation of Dr Toshikazu Yoshikawa, a leading researcher on oxidative stress, Dr Hiroshi Ichikawa of Doshisha University School of Life and Medical Sciences and Dr Shigekazu Takemura of Osaka Metropolitan University Graduate School of Medicine, with whom I have worked for many years, and many other collaborators. For this I am very grateful,' highlights Minamiyama.

> *"We would like to share with the world the means to prevent the effects of the chemicals that humans ingest during their lifetime"*

OXIDATIVE STRESS AND ANTIOXIDANTS

Oxidative stress is an imbalance in levels of oxidants aka free radicals and antioxidants that can otherwise be described as excess production of reactive oxygen species (ROS) compared with antioxidant defence and when it reaches chronic levels this can lead to various diseases. 'Food additives are unavoidable, but chronic oxidative stress can cause infertility, cerebrovascular disease and juvenile dementia,' explains Minamiyama. 'We believe that controlling free radicals and ROS can prevent or improve diseases.' As it would be extremely difficult to avoid additives, Minamiyama believes that if antioxidants are simultaneously consumed, this can reduce oxidative stress and the resultant diseases. In one study the team looked into food additive-induced oxidative stress in rat male reproductive organs and the hippocampus. The full extent to which food additives and other chemicals such as those from packaging and pesticides can affect health and reproduction is unknown and in order to fill this knowledge gap, they investigated the *in vitro* and *in vivo* effects of food additives on the male rat brain and sperm/testes, with an emphasis on oxidative stress. 'We found that stevioside, which is a food additive found in the sweetener stevia, along with other preservatives, induced ROS production in sperm and resulted in sperm dysfunction,' describes Minamiyama. 'Furthermore, we discovered that treatment with an antioxidant called alpha-tocopherol significantly improved oxidative stress; a finding that suggests that antioxidants may have the potential to prevent the oxidative stress induced by food additives,' she says. In another line of investigation, the

researchers are exploring the actions of various sweeteners on rat sperm. The goal is to study the theory that male infertility may be caused by dysfunction due to excessive ROS production from sperm mitochondria. In this work, they explored the action of various common food additive sweeteners on rat sperm *in vitro* and confirmed that some sweeteners affect sperm.

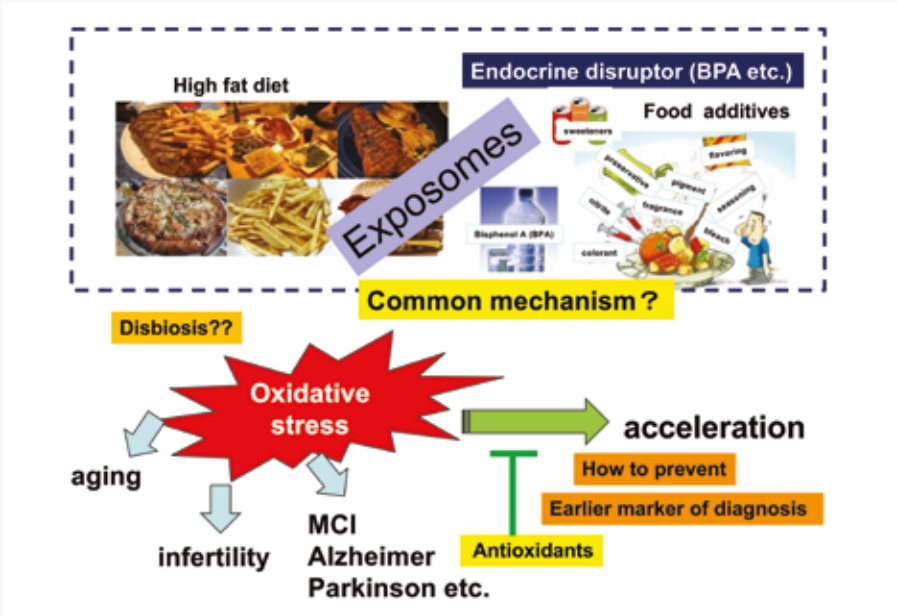
ROS AND TOXICITY

Another branch of Minamiyama's investigations is the generation of ROS in sperms of rats as an earlier marker for evaluating the toxicity of endocrine-disrupting chemicals. The researchers were interested in deciphering whether levels of ROS in sperm could predict sperm toxicity and quality. 'To test this theory, they administered endocrine disruptors bisphenol A (BPA) and diethylstilbestrol (DES), which have been reported to cause sperm toxicity, to rats and measured the resulting impact on sperm,' clarifies Minamiyama. 'It was found that endocrine disruptors increase ROS production from sperm prior to a decrease in sperm count or motility, and that excess ROS production occurs even with lower concentrations of endocrine disruptors.' She explains this indicates that prevention is possible by administering antioxidants or stopping the intake of chemicals before infertility or obvious symptoms develop.

In unpublished work, the researchers are looking at biological impact of additives, for example, tau protein phosphorylation and Alzheimer's disease using a model they have developed. 'It is believed that the tau protein aggregation seen in Alzheimer's disease is caused by phosphorylation of tau protein at more than a few sites, and we have found at least one site of tau phosphorylation in this model of ours,' Minamiyama reveals. 'Therefore, we believe that long-term consumption of such food additives may hasten the onset of dementia, and indeed the WHO reports that dementia is increasing and occurring at younger ages.'

An important goal for Minamiyama is to detect changes in the oxidation and phosphorylation of certain proteins in the testes. As the testes and the brain produce similar proteins, this may be a route to predicting changes in the brain. 'Endocrine organs, such as the testes and the brain, are very similar proteins they express, and their metabolism is different from that of the liver and other organs, with a mechanism that protects them even under starvation conditions,' she explains. 'The testes also express many of the tau proteins expressed in the brain in Alzheimer's disease and PARK-7 expressed in Parkinson's disease, and it can be said that the brain and sperm (testes) are very similar.' Therefore, if changes can be detected in the oxidation and phosphorylation of these proteins in the testes, they may be able to predict changes in the brain.

From Minamiyama's studies to date, the idea that antioxidants could inhibit ROS production and help maintain wellbeing is supported. 'If excessive ROS production and resulting oxidative stress are caused by food additives and other chemicals, I would like to be able to announce that I believe that taking food and supplements that show antioxidant activity at the same time can prevent diseases that may occur in the future,' Minamiyama states. Pending future studies surrounding the concept of the exposome, she hopes that her research findings can assist with maintaining health, as she underlines: 'We would like to share with the world the means to prevent the effects of the chemicals, namely exposomes that humans ingest during their lifetime.' This could equip humankind with the tools needed to maintain health and wellbeing, slowing or even preventing the onset of many diseases.



The exposome refers to the totality of exposures (both external and internal) to which an individual is subjected to their entire life. Exposomes accelerate various diseases such as infertility, Mild Cognitive Impairment (MCI), Alzheimer's, Parkinson's and ageing via oxidative stress. Intake of antioxidants may stop the acceleration.

Project Insights

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BIO

Dr Yukiko Minamiyama has been a Professor at the Department of Food Environment and Safety, Division of Applied Life Sciences, Graduate School of Life and Environmental Sciences, Kyoto Prefectural University since 2010, where she is currently the Vice President. She has a particular interest in pathological biochemistry, free radicals and ROS-related disease. Good lifestyle habits and antioxidant intake can reduce most their diseases such as atherosclerosis, cancer, diabetes mellitus, stroke and ageing.