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学位論文全文に代わる要約
Extended Summary in Lieu of Dissertation

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Name

学位論文題目：　　Studies on functional hexoses with anti-aging effects
Title of Dissertation　　（抗老化効果を有する機能性ヘキソースに関する研究）

学位論文要約：
Contents

Part 1. General introduction

The emperor in China is reported to have ordered a search for an elixir of immortality. Moreover, in another legend, Egyptian pharaohs desired an anti-aging medicine. Even in Japan, the global leader in longevity, new information on health and longevity is easily accessible from the media because people seek knowledge on this subject.

In the 2000s, calorie restriction (CR) was shown to extend the lifespan of rhesus macaques, which are primates [1]. However, a subsequent report [2] indicated that CR promoted health but did not extend lifespan. Because these studies did not use the same dietary conditions, it was difficult to directly compare the results [3]. Additionally, in primates, CR was suggested to extend lifespan depending on dietary conditions. In recent years, studies have progressed to searching for and evaluating compounds showing effects similar to those of CR following oral administration.

CR has been shown to prolong the lifespan of experimental animal models, such as nematodes, flies, and mice. Additionally, CR delays the progression of diverse age-related changes and diseases. The Comprehensive Assessment of Long-Term Effects of Reducing Intake of Energy (CALERIE) study was the first study focused on the effects of CR in humans [4,5]. The CALERIE study demonstrated the feasibility of CR in humans and its favorable effects on predictors of longevity and cardiac metabolic risk factors [6]. The CALERIE trial was conducted in two stages. Phase 1 was a preliminary study for determining the target percentage of CR, whereas phase 2 tested the effects of CR in a 2-year randomized clinical

trial. The longer intervention period resulted in an extended CR effect that persisted after the acute effects of CR on weight loss. The purpose of the CALERIE phase 2 trial was to test the hypothesis that human CR causes persistent metabolic adaptation. The phase 2 trial was a 2-year three-site randomized controlled trial in young and middle-aged nonobese healthy women and men. As a result, in the CR group, weight loss, body fat reduction, fluctuations in energy consumption, and decreased oxidative stress markers were observed [7]. These changes were also observed in experimental animal models of CR.

Even if CR prolongs the human lifespan, it is difficult to enforce long-term CR in humans. Therefore, it is preferable to develop an alternative method or drug/ dietary supplement that can reproduce the effects of CR without limiting food intake. The concept of CR mimetics (CRMs) was proposed by Lane et al. [8] in a study of D-glucose analog, 2-deoxy-D-glucose (Figure 1), which showed bioactivity in rats. CRMs exhibit the systemic effects of CR and broadly include not only compounds but also methods, such as bariatric surgery or exercise [7,9,10].

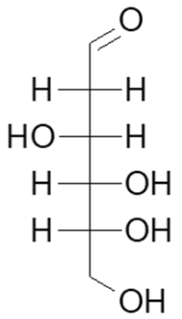

Figure 1. Molecular structure of 2-deoxy-D-glucose

Table 1. Downstream-type CRMs.

Compound	Mode of action
Metformin (antidiabetic drug)	AMPK activation
Rapamysin (immunosuppresant drug)	mTOR inhibition
Resveratrol (food component)	Sirtuin activation
Polyamines (food component)	Epigenetic control
Oxaloacetic acid (dietary supplement)	Redox balance

Downstream and upstream CRMs have been identified [9, 10]. Downstream-type CRMs act on intracellular signaling systems and exert the same effects as CR on downstream pathways (Table 1). In contrast, an upstream-type CRM uses a mechanism of action targeting the energy metabolism system and transmitting a signal in the upstream direction to mimic CR (Table 2).

Table 2. Upstream-type CRMs.

Compound	Mode of action
Chitosan (dietary supplement)	Glucose diminution
Acarbose (antidiabetic drug)	Glycosidase inhibition
2-Deoxy-D-glucose (anticancer drug)	Glycolysis inhibition
SGLT2 inhibitor (antidiabetic drug)	Glucose excretion

In nature, carbohydrates exist as polysaccharides, such as chitin, chitosan, cellulose, and starch. Hexoses can be produced at an industrial scale for use in foods from polysaccharides that are abundant in nature and available at low cost. Therefore, identification of compounds with high value-added health functions such as CR among hexoses may facilitate the improvement of quality of life via consumption as foods or functional foods.

In this part, I described the application of upstream-type CRMs as compounds that inhibit energy metabolism, particularly D-glucose metabolism, and focus on D-glucose analogs. In the next part, I will expand on these topics.

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Part 2. Overview of hexoses production

Most monosaccharides in nature are hexoses, which have six carbon atoms; the most well-known hexose is D-glucose (Figure 1.). Various hexoses with distinct characteristics can be produced from inexpensive polysaccharides for applications in the food industry. Therefore, identification of the health-related functions of hexose will facilitate the consumption of hexoses in food products to improve quality of life. The hexoses available in foods include *N*-acetyl glucosamine, D-glucosamine, D-fructose, D-mannose, D-galactose, other D-hexoses, and l-hexoses (Figure 2.). Here, an updated overview of food industrial production methods for natural hexoses by microbial, enzymatic, and chemical methods is provided.

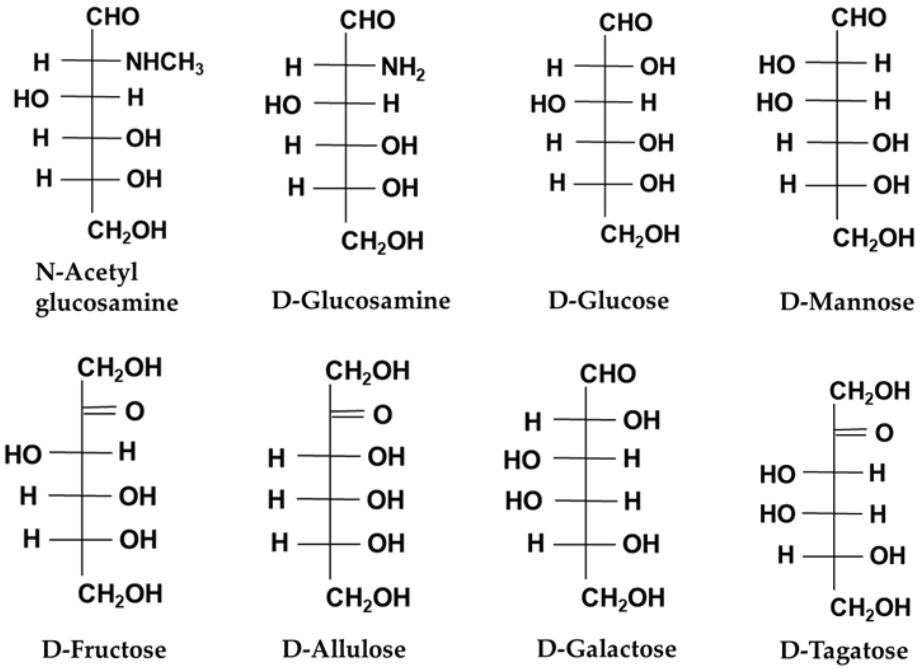

Figure 1. Molecular structures of D-hexoses

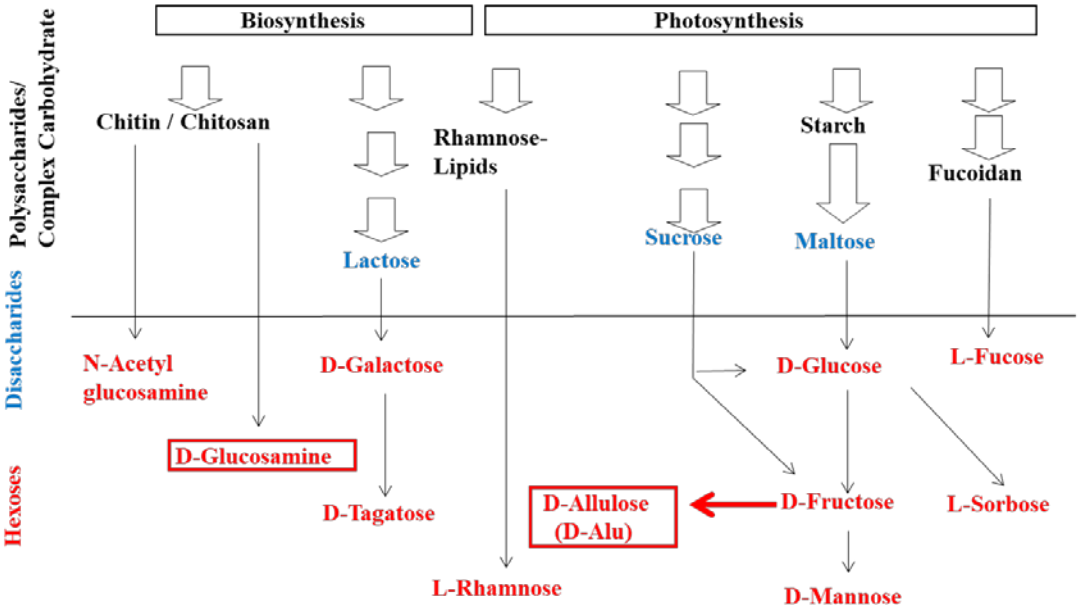

Figure 2. Various hexoses and those origins

Part 3. Production of D-allulose

Arthrobacter species have long been used in the food industry and are well known for their high degree of safety. In this study, an enzyme that catalyzes C-3 epimerization between D-fructose and D-allulose was found in *Arthrobacter globiformis* strain M30. The purified enzyme was characterized as a D-allulose 3-epimerase. The enzyme showed maximal activity and thermostability in the presence of Mg^{2+}. The optimal pH and temperature for enzymatic activity were 7.5- 8.0 and 70℃, respectively. The enzyme was immobilized to ion-exchange resin and was then stable for longer periods than the free enzyme when stored at below 10℃. In the column reaction, the enzyme activity also maintained stability for more than 4 months. Under these conditions, 215 kg D-allulose was produced per liter of immobilized enzyme, and this was the highest production yield of D-allulose reported to date. These highly stable properties suggested that this enzyme represented an ideal candidate for the industrial production of D-allulose.

Part 4. Evaluation of hexoses and D-glucosamine in vitro: autophagy activity

Autophagy is a cellular process that nonspecifically degrades cytosolic components and is involved in many anti-aging cellular responses (Figure 1.). We found that amino sugars with a free amino group, such as D-glucosamine (D-GlcN), D-galactosamine, and D-mannosamine, induced autophagy via a mammalian target of rapamycin-independent pathway (Figure 2.). D-GlcN-induced autophagy at concentrations of at least 500 μM to over 40 mM. In the presence of 40 mM glucosamine, autophagy induction was initiated at 6 h and reached a plateau at

36 h. D-GlcN-induced autophagy could remove accumulated ubiquitin-conjugated proteins as well as 79-glutamine repeats. Therefore, orally administered D-GlcN could contribute to the prevention of neurodegenerative diseases and promotion of anti-aging effects.

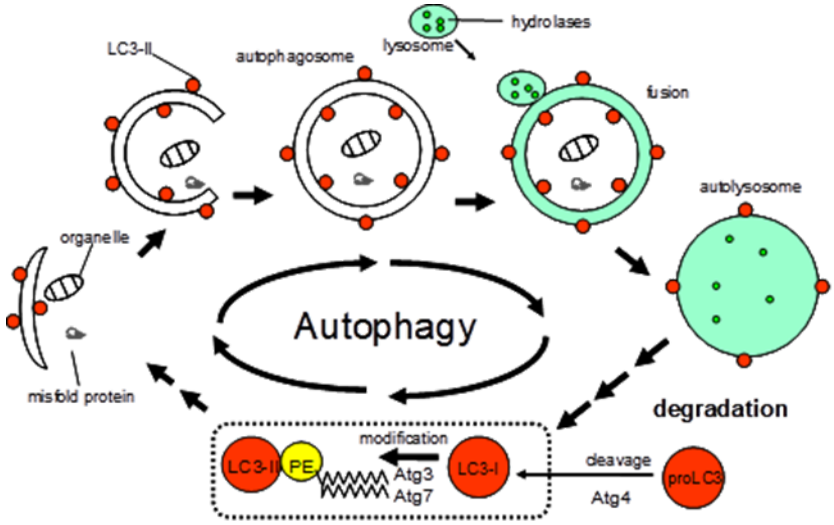

Figure 1. Autophagy cycle

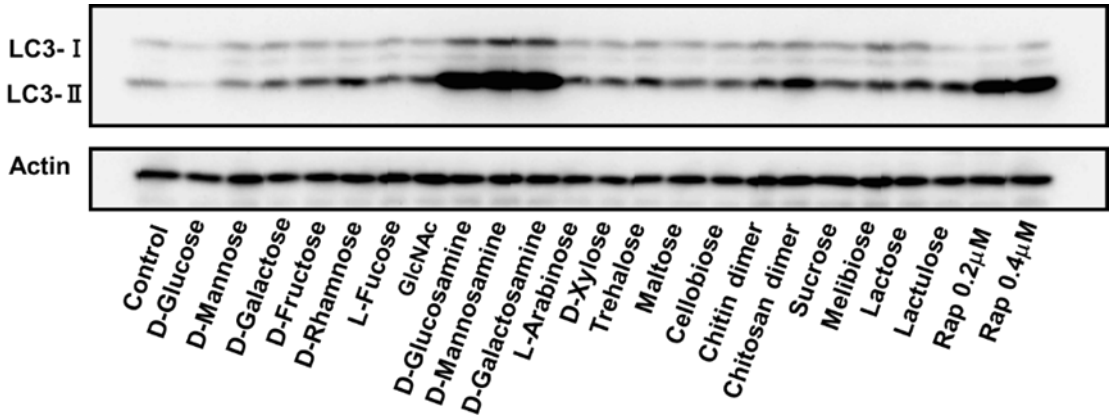

Figure 2. D-GlcN induced autophagy in mammalian cells

Part 5. Evaluation of D-glucosamine in nematodes: longevity activity

D-Glucosamine (D-GlcN) is a commonly used dietary supplement that may promote cartilage health in humans. We previously reported that D-GlcN induces autophagy in cultured mammalian cells. Autophagy is known to be involved in prevention of various diseases and aging. In this study, we evaluated the role of D-GlcN in increasing longevity. Our results showed that D-GlcN extended the lifespan of the nematode *Caenorhabditis elegans* by inducing autophagy. Autophagy induction in nematodes by D-GlcN was confirmed by western blotting of LGG-1, an ortholog of mammalian LC3, and by detection of autophagosomal dots using fluorescent microscopy (Figure 1.). In lifespan assays, D-GlcN-induced lifespan

extension was observed after treatment with at least 5 mM D-GlcN. The maximum lifespan extension was observed at 20 mM D-GlcN. D-GlcN-induced lifespan extension was not dependent on the general longevity genes, *daf-16* and *sir-2.1*, but was dependent on the *atg-18* gene, which is essential for autophagy. Therefore, our findings suggested that oral administration of D-GlcN could delay aging via induction of autophagy.

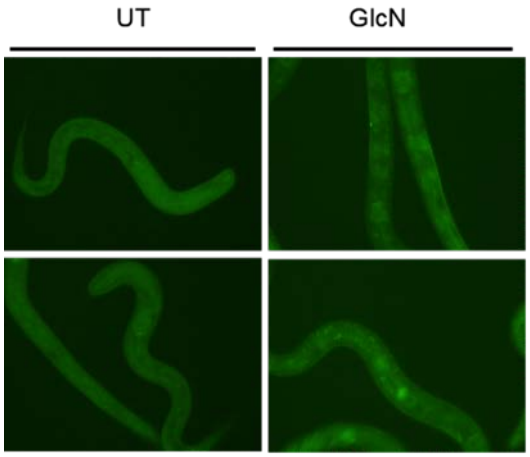

Figure 1. D-GlcN induced autophagy in *C. elegans*.

Part 6. Evaluation of D-allulose in nematodes: longevity activity

Dietary restriction (DR), including calorie restriction, is known to increase lifespan in various organisms and delays the onset of aging-associated diseases. DR mimetics have been extensively explored. D-Allulose (D-Alu), the C3-epimer of D-fructose, is a rare sugar that has various health benefits, including antihyperglycemic and anti-obesity effects. Here, we examined the effects of D-Alu on the lifespan of *Caenorhabditis elegans* under some culture conditions. D-Alu did not further extend the lifespan of the long-lived DR model *eat-2* mutant, strongly indicating that the effect was related to DR. However, D-Alu did not reduce food intake of wild-type *C. elegans*. To explore the mechanisms through which D-Alu extended longevity, we examined the lifespan of D-Alu-treated mutants deficient for the nutrient-sensing pathway-related genes *daf-16*, *sir-2.1*, *aak-2*, and *skn-1*. The results showed that D-Alu increased the lifespan of the *daf-16*, *sir-2.1*, and *skn-1* mutants, but not the *aak-2* mutant, indicating that the lifespan extension was dependent on the energy sensor, AMP-activated protein kinase. D-Alu also enhanced the mRNA expression and enzyme activities of superoxide dismutase and catalase. From these findings, we concluded that D-Alu extended lifespan by increasing oxidative stress resistance through a mechanism involving DR, making D-Alu a candidate DR mimetic.

Part 7. Evaluation of D-allulose in the modulation of rodent glucose metabolism

Ingestion of high-fructose corn syrup (HFCS) is associated with diabetes and obesity. Rare sugar syrup (RSS) was developed by alkaline isomerization of HFCS to contain

D-allulose and has anti-obesity and antidiabetic effects. However, the influence of RSS on glucose metabolism is unclear. Here, we investigated whether long-term administration of RSS maintained glucose tolerance and whether the underlying mechanisms involved hepatic glucokinase translocation. Wistar rats were administered water, RSS, or HFCS for 10 weeks and then evaluated. RSS significantly suppressed body weight gain and abdominal fat mass. Additionally, glucose tolerance tests revealed significantly higher blood glucose levels in the HFCS group than in the water group, whereas the RSS group had significantly lower blood glucose levels. The amount of hepatic glycogen was more than three times higher in the RSS group than in the other groups. After glucose loading, glucokinase nuclear export was significantly increased in the RSS group compared with that in the water group. These results implied that RSS maintained glucose tolerance and insulin sensitivity by enhancing the nuclear export of hepatic glucokinase.

Part 8. Application of D-allulose for modulation of glucose metabolism in humans

Many studies have shown that ingestion of added sugars, such as sucrose and high-fructose corn syrup, may cause metabolic syndrome risk factors. A new sweetener containing rare sugars such as D-allulose, called rare sugar syrup (RSS), has been reported as an anti-obesity sweetener. Here, the effects of RSS on blood glucose levels in healthy participants were investigated in a controlled crossover trial. In this experiment, the glycemic indexes of sucrose and RSS were found to be 64 and 49, respectively. These results showed that RSS was a low glycemic index sweetener and that the use of RSS had blood glucose-lowering effects.

Part 9. General Discussion

Since ancient times, human beings have sought longevity. Lane et al. [1] proposed the calorie restriction (CR) mimetic (CRM) concept in 1998. Studies of CRMs have progressed greatly in the past 20 years. Moreover, new candidates, including D-allulose (D-Alu) and D-glucosamine (D-GlcN), both of which are functional hexoses with high safety and health benefits, have been shown to affect longevity.

D-GlcN is a constitutional unit of chitosan and chitin, which are produced in nature by arthropods, fungi, and cephalopods. D-GlcN is industrially manufactured by the hydrolysis of crustacean exoskeletons, which are mainly composed of chitin. D-GlcN is a popular dietary supplement that effectively prevents and treats osteoarthritis in humans [2].

Recently, Weimer et al. [3] reported the effects of D-GlcN on longevity in mice. The authors suggested that these effects were caused by impaired glucose metabolism. In contrast, We showed that the effects of D-GlcN on longevity required an autophagy gene; however, the longevity genes *sir-2.1* and *daf-16* were not required for D-GlcN-induced lifespan extension, unlike that induced by other CRMs and CR. Similar to 2-deoxy-D-glucose (2-DG), D-GlcN enters cells through hexose transporters and inhibits glycolysis, inducing the metabolism of stored fat and mitochondrial respiration via AMP-activated protein

kinase (AMPK). Increased respiration can cause formation of reactive oxygen species (ROS), leading to increases in antioxidative enzyme activity, oxidative stress resistance, and survival rates [4,5]. Orally administered D-GlcN has also been reported to influence carbohydrate metabolism and reduce body fat in rodents [6] and contribute to enhanced oxidative stress resistance, followed by AMPK activation [3]. The compound has also been reported to induce autophagy in mammalian cells via a mammalian target of rapamycin-independent signaling pathway [7]. Therefore, the mechanisms through which D-GlcN exerts anti-aging effects may be similar to those of 2-DG.
In a clinical trial, oral administration of D-GlcN improved vascular endothelial function by modulating the intracellular redox state [8]. Moreover, according to a large-scale epidemiological study on consumers of various dietary supplements, the use of D-GlcN was associated with a decrease in total mortality [9].

D-Alu (also known as D-psicose), a C-3 epimer of D-fructose, is a rare hexose sugar present in a limited quantity in nature. However, this compound is marketed as a functional sweetener with zero calories [10] and is easy to produce at high yields from D-fructose [11]. In the past decade, numerous studies have shown that D-Alu exhibits various activities, such as antihyperglycemic and anti-obesity effects [12]. Recently, Shintani et al. [13] reported that long-term administration of a rare sugar syrup containing D-Alu maintained glucose tolerance and insulin sensitivity in rats via hepatic glucokinase translocation. Thus, D-Alu is expected to be a potent antidiabetic and anti-obesity sweetener. We also reported that a high dose of D-Alu suppressed increases in body size during the young adult stage of the nematode *Caenorhabditis elegans* [14]. Recently, D-Alu was reported to extend the lifespan of nematodes [15]. Similar to D-GlcN and 2-DG, D-Alu enters cells through glucose transporters and inhibits glycolysis, thereby inducing the metabolism of stored fat and mitochondrial respiration via AMPK. Increased respiration causes temporary upregulation of ROS production, leading to increased antioxidant activity, oxidative stress resistance, and survival rates [4,5,15]. Body fat reduction was observed in *C. elegans* [15]. More recently, D-Alu was reported to suppress carbohydrate oxidation and promote fat oxidation in rodents [16], supporting the above *C. elegans* model data [15].

In a clinical trial, D-Alu was shown to be a CRM based on changes in biomarker levels, such glucose and body fat. Clinical trials using a maltodextrin diet or standard meal confirmed that D-Alu suppresses postprandial blood glucose levels [17,18]. Moreover, syrup containing D-Alu showed a low glycemic response in healthy humans [19]. Even a single dose of D-Alu was reported to enhance postprandial fat oxidation in healthy humans [20]. Upon continuous intake of D-Alu, the percentages of body fat and body fat mass decreased significantly, with no significant reduction in nutrient intake [21].

Commonalities have been observed between CR and upstream-type CRMs, revealing similar effects on selected biomarkers in experimental animal models and humans. However, additional studies are needed to determine the mechanisms and efficacies of CRMs. CRM candidate substances that have already been studied or may be identified in the future should meet certain requirements. In particular, it is necessary to confirm the effects of compounds on lifespan without CR and elucidate the mechanisms of action of these compounds as CRMs in model animals. The findings will eventually have to be confirmed in humans.

The functions of foods and medicines have been explored not only to test the efficacy of foods and medicines but also to promote health through basic food research, which could lead to a healthily aging community. The most trusted method for healthy aging is dietary restriction or CR [22]. CR has been shown to prolong the lifespan of experimental animals, such as nematodes, flies, and mice. In a substantial clinical study, CR was reported to delay the progression of diverse age-related changes. Even if CR prolongs the human lifespan, it is difficult to conduct long-term CR experiments in humans. Therefore, it is preferable to develop a method or compound that reproduces the effect of CR without limiting dietary intake. Recent research has showed that CRMs are expected to have efficacy against age-related diseases, such as cardiovascular and neurodegenerative diseases [23]. The CRMs resveratrol, spermidine, oxaloacetic acid, D-GlcN and other compounds are food components that exhibit anti-aging and longevity-promoting effects (Figure 1.) [22]. Some CRMs are made from petrochemicals; hence, they may not be practical for human use. However, food-derived components will most likely be readily applicable.

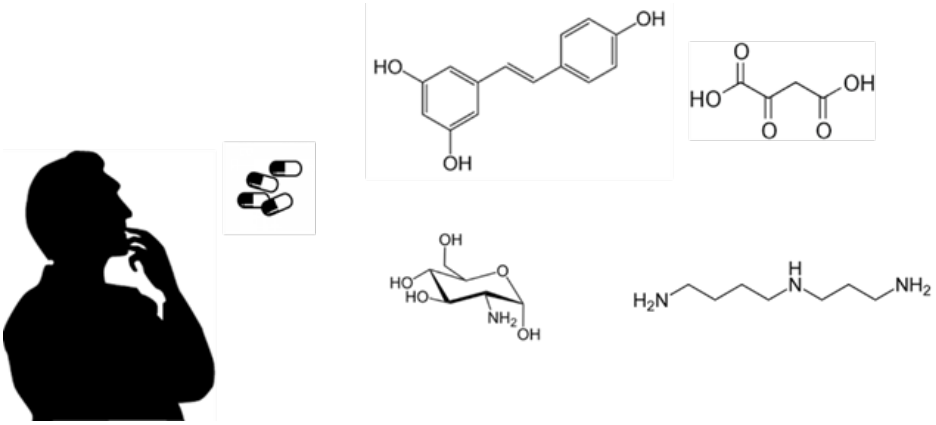

Figure 1. Humans and CRMs.

Life sciences researchers should further contribute to the field of CRMs, and improving our understanding of the chemical properties of food-derived CRMs is essential for increasing their applications. For example, CRMs that do not undergo changes or oxidation easily under normal temperature, normal pressure, and low oxygen concentration should be researched. Such substances will have a low final cost owing to their stable properties. Furthermore, these potential CRMs should be easy to access and produce, allowing them to be made readily available in the market. The methods for the production of substances, such as functional hexoses (e.g., D-GlcN and D-Alu), are being researched and developed; however, further research on production methods is still required for many other compounds. Thus, although the function of CRMs has been established, their practical application will not be possible until the chemical properties of CRMs are understood and until these compounds can be easily produced. Furthermore, applied research on processed foods containing CRMs and on CRMs alone is also extremely important for improving health and longevity in our society. Although excessive dietary intake has been reported to impair health and cause obesity and diabetes worldwide, it is difficult to reduce daily dietary intake immediately and prevent lifestyle-related diseases. It is relatively easier for most people to consume additional foods, dietary supplements, or medicines derived from foods. Further studies are needed to research for practical antiaging applications.

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General introduction, Part 2 and General discussion in this extended summary contain a part of modified versions of the following two articles.

Shintani, H.; Shintani, T.; Ashida, H.; Sato, M. Calorie restriction mimetics: upstream-type compounds for modulating glucose metabolism. *Nutrients* 2018, *10*, doi:10.3390/nu10121821.

Shintani, T.; Food industrial production of monosaccharides using microbial, enzymatic, and chemical methods. *Fermentation* 2019, *5*, doi:10.3390/fermentation5020047.