Preventive Effects of Health-Food Drinks on the Obesity and DNA Damage of Mice Fed a High-Fat Diet under a Mild Stress

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Abstract: The body weight gain of mice fed an obesity-inducing diet is suppressed by a mild restraint stress, but at the same time, as previously reported, the stress induces DNA damage in the cells of multiple organs. In the present study, we attempted to prevent not only the obesity but also the DNA damage of mice fed an obesity-inducing diet under a mild restraint with commercially available health-food drinks such as fruit and vegetable juices, soymilks, vinegars and lactic acid bacteria drinks, which are rich in antioxidants. The body weight gain of young female mice fed a high-fat diet containing 20% fat for 4 weeks was considerably inhibited by restraint for 15 min per day in weeks 2 to 4 of 4-week period. The inhibition was further promoted with the concomitant administration of health-food drinks noted above, and was accompanied by a decrease of periovular fat, a major abdominal fat in the female mice. The definite loss of energy intake in the mice given health-food drinks was approximately compensated by the energy of the drink administered. On the other hand, the increase of DNA damage generated by restraint in the cells of five organs – the liver, pancreas, spleen, heart and bone marrow – was markedly suppressed with the administration of these drinks. The results suggest that a combination of mild stress and intake of suitable health-food containing some antioxidants may inhibit lifestyle-related diseases including hyperlipidemia and obesity, which may contribute to the inhibition of metabolic syndrome and childhood obesity.

INTRODUCTION

It has been shown that mental and physiological stresses induce excess reactive oxygen species (ROS), worsen lifestyle-related diseases, cause cellular damage via oxidation of biomolecules, including DNA, proteins and polyunsaturated fatty acids, and finally induce a variety of diseases such as cancer, Parkinson's disease, and cardiovascular diseases [1-4]. In addition, unhealthy lifestyles such as excessive intake of high-fat diet and lack of exercise are prevalent among people today, and they can induce, promote and increase the lifestyle-related diseases [5-9]. The authors have been studying biological antioxidant defense systems induced by a variety of stresses and the antioxidant effects against reactive oxygen species [10-15], and are currently interested in the contribution of biological antioxidant defense system and food to the relationship between obesity and stress, investigating the possibility of utilizing beneficial effects of mild stress [7, 16]. We previously reported that food-induced dyslipidemia in mice could be accelerated by repeated fasting, but could be suppressed by repeated restraint, and that the DNA damage in cells of various organs was increased by repeated stress [13, 15]. In these studies, we investigated the DNA damage in the cells of four murine organs susceptible to the influence of stress, using mild restraint stress. While some benefits of stress including suppression of body weight gain, improvement of fatty liver and activation of biological defense systems were observed, mild DNA damages were also detected in the cells of murine organs. Mild and beneficial stress is known to activate the brain and promote our health, especially in the elderly [1, 3]. In the present study, we aimed to investigate whether a variety of health-food drinks commercially available may be able to prevent or reduce mild DNA damage in the cells with their antioxidative effects [2, 17, 18], in combination with mild stress.

EXPERIMENTAL METHODS

Animal Treatments

Female weaning-age ICR mice (Japan SLC, Inc., Shizuoka, Japan) were divided into four to six groups as shown in Table **1**. All of the experiments were performed in duplicate (n=12). The mice assigned to the stress groups were fed high-fat diet (HFD) for 4 weeks and were subjected to fasting for 24 hours from 20:00 every other day, three times per week (F24), or to restraint with a tube for 15 min (R15) daily on weekdays, in weeks 2 to 4 of the 4-week study period as shown in the previous paper [13]. Control mice were fed standard diet (STD) (C1) or HFD (C2) for 4 weeks

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Table 1: Experimental Design

Animal	Female ICR mice, 4 weeks of age, n=6	
Health-food drink group	1. Fruit and vegetable juice (12) Fv1-12 (40-65% fruit juice + 35-60% vegetable juice)	
	2. Other Health-food drink (14) Hd1-3 (soy milks),Hd4-7 (black vinegars), Hd8-10 (fruit juices), Hd11-14 (lactic acid bacteria beverages)	
Control group	C1. standard diet	Standard diet control
	C2. high-fat diet	High-fat diet control
	F12. 12-h fasting	Obesity control
	F24. 24-h fasting	Dyslipidemia control
	15-min restraint	Restraint control
Fat composition of diet	Standard diet	Soy oil 4.4%
	High-fat diet	Beef tallow 13% Corn oil 9.6% Cholesterol 1.0%
Treatment period	Feeding period	4 weeks
	Restraint period	Weeks 2-4

without being subjected to any stress. The HFD (Crea Japan, Osaka, Japan), specially prepared for our laboratory, was based on the AIN 76 diet [19], containing 9.6% corn oil, 13% beef tallow, 1% cholesterol, 0.2% cholic acid, and 433.0 kcal/100 g. The STD (CE-2) containing 4.4% fat and 342.2 kcal/100 g was purchased from Crea Japan. These solid-type diets were weighed and fed adequately. Total energy intakes were calculated as the sum of diet eaten by each treatment groups during the experiment. Cages of the animals were cleaned regularly during the fasting period to prevent coprophagy. Restraint was performed using a handmade restraint device, a 50-ml plastic centrifuging tube modified for this experiment, as shown in the previous paper [15]. The device has twelve 3-mm diameter holes for sweating, one hole for tail-protecting, and four holes fitted with two steel rings for the animal's habitual teeth grinding and breathing. Volume of centrifuging tube was changed in accordance with the size of the animal. At the end of the treatment period, mice exposed to stress were fed HFD for approximately one day, and all the animals were fasted for 2 hours before they were sacrificed. All experimental procedures were approved by the Animal Care and Use Arrangements Meetings of Suzugamine Women's Coll. and of Nagoya Keizai Univ. and conformed to the guidelines established by the Japanese Ministry of Education, Culture, Sports, Science and Technology.

Sample Preparation

At the end of the 4-week study period, blood samples were collected from the supraorbital vein of the animals under pentobarbital anesthesia. Then, the liver, pancreas, spleen and heart were quickly removed from each animal, weighed and placed on ice. The bone marrow cells were collected from the femur and eluted with phosphate buffered saline.

Comet Assay

Aliquots of mouse organs (liver, pancreas, spleen and heart) were homogenized in glass-teflon homogenizers. The homogenates and bone marrow eluate were subjected to the Comet assay [20, 21] using the Comet Assay and Silver Staining Kits (Trevigen, Gaitherburg, MD, USA) and SYBR Gold (Invitrogen, Eugene, OR, USA). The comet images obtained by using an optical or a fluorescence microscope equipped with a digital camera were analyzed and were scored using a comet analysis software (CometAnalyzer, Youworks, Tokyo, Japan). The DNA damage was expressed as a ratio of the total comet intensity to the head intensity of DNA image from 100 cells of each sample.

Statistical Analysis

Data are presented as mean (± SEM). Differences in means were analyzed using unpaired *t*-test with

InStat 2.0 (GraphPad Software Inc.). Statistical significance was defined as *p*< 0.05.

RESULTS

Body Weight Change

Body weights of control mice in the HFD control group (C2) and the 24-h fasted group (F24) were similarly increased and were higher than those of mice in the STD control group (C1), and those in the 12-h fasted group (F12) were highly increased as shown in Figure **1**. On the other hand, the increase in body weight of the restraint group (R15) was clearly reduced by stress as shown in Figure **1**. Body weight gains of mice in most of the twelve experimental drink groups (Fv1-12) were reduced to the level lower than those of the restraint group as shown in Figure **1**. Similar tendencies were seen in the body weights of mice administered with the other fourteen health-food drinks as shown in Figure **2** (Hd1-14).

Abdominal Fat Weight

Weights of the periovular fat which is a major abdominal fat of female mice were increased in the
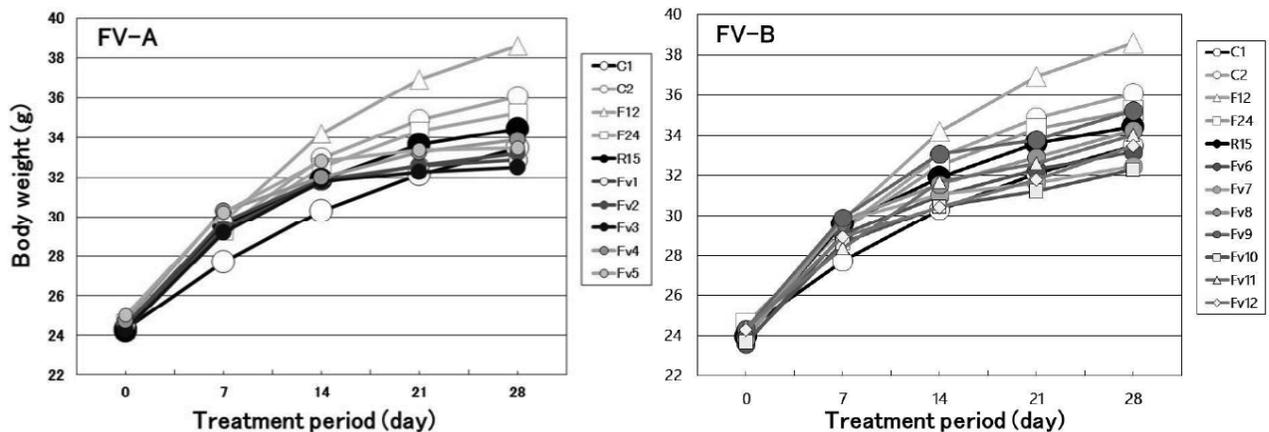
Figure 1: Change in body weight in mice of the control groups and the experimental drink groups exposed to restraint repeatedly for 15 min per day under concomitant administration of high-fat diet and one of twelve fruit and vegetable juices.

C1, standard diet control; C2, high-fat diet control; F12 and F24, high-fat diet plus fasting for 12 h and 24 h, respectively; R15, high-fat diet plus restraint for 15 min. Mice were exposed to stress daily on weekdays in weeks 2 to 4 of the 4-week study period, except for F24 (every other day) mice. Data of the control groups (C1, C2, F12, F24 and R15) and the experimental drink groups are presented as means obtained from two experimental groups for twelve fruit and vegetable juice groups (FV-A: Fv1-5 and FV-B: Fv6-12), n=12.
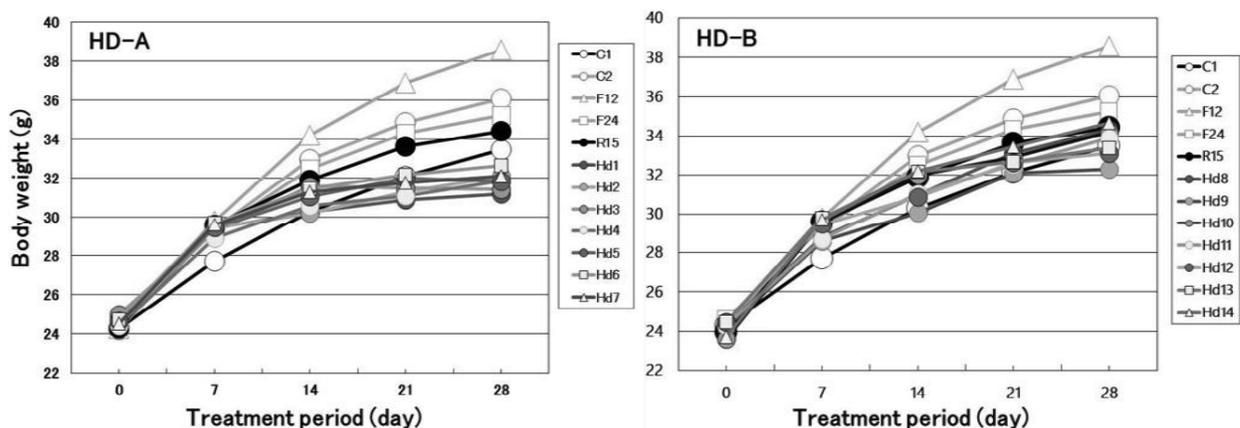
Figure 2: Change in body weight in mice of the control groups and the experimental drink groups exposed to restraint repeatedly for 15 min per day under concomitant administration of high-fat diet and one of the other fourteen health-food drinks.

C1, standard diet control; C2, high-fat diet control; F12 and F24, high-fat diet plus fasting for 12 h and 24 h, respectively; R15, high-fat diet plus restraint for 15 min. Mice were exposed to stress daily on weekdays in weeks 2 to 4 of the 4-week study period, except for F24 (every other day) mice. Data of the control groups (C1, C2, F12, F24 and R15) and the experimental drink groups are presented as means obtained for the other fourteen health-food drink groups (HD-A: Hd1-7 and HD-B: Hd8-14), n=12.
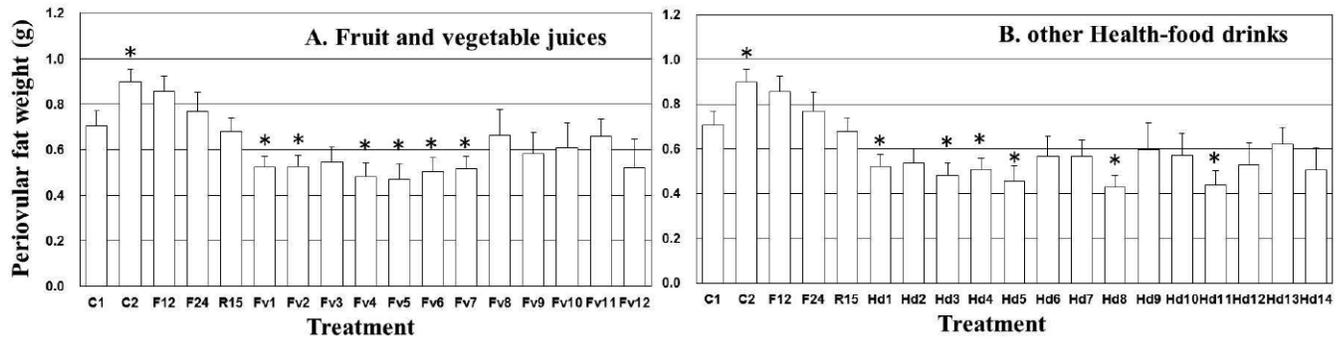
Figure 3: Periovular fat weight in mice of the control groups and the experimental drink groups exposed to restraint repeatedly for 15 min per day under concomitant administration of high-fat diet and one of twelve fruit and vegetable juices and the other fourteen health-food drinks.

C1, standard diet control; C2, high-fat diet control; F12 and F24, high-fat diet plus fasting for 12 h and 24 h, respectively; R15, high-fat diet plus restraint for 15 min. Mice were exposed to stress daily on weekdays in weeks 2 to 4 of the 4-week study period, except for F24 (every other day) mice. Data of the control groups (C1, C2, F12, F24 and R15) and the experimental drink groups are presented as mean± SEM obtained from twelve fruit and vegetable juice groups (A. Fv1-12) and from the other fourteen health-food drink groups (B. Hd1-14), n=12. . * *p*< 0.05 vs. corresponding R15 group.

HFD control group and the fat weights were gradually reduced in proportion to the intensity of the stress and the fat weights of mice in the restraint control group were reduced to the same level as those of the standard diet group as shown in Figure **3**. The fat weight was further reduced by the concomitant administration of most kinds of the twelve fruit and vegetable juices and the other health-food drinks similarly, as shown in Figure **3**. There were significant differences between the weights for 50% of the fruit and vegetable juice groups and for 43% of the other health-food drink groups and that of the restraint control group.

The weights of other organs, that is, liver, pancreas, spleen and heart, did not show significant differences

between the health-food drink groups and the restraint control group (data not shown).

Total Energy Intake

Total energy intake in each experiment group was expressed as the percentage to the total energy intake in the corresponding STD control group (C1) as shown in Figure **4**. Relative energy intake of the HFD control group (C2) was higher than that for the corresponding STD control group. Both in the fruit and vegetable juice group and the other health-food drink group, total energy intakes of 12- and 24-h fasting (F12 and F24) and restraint (R15) groups were almost similar to that of the corresponding STD control group (C1). Although the energy intakes from diet in the fruit and vegetable
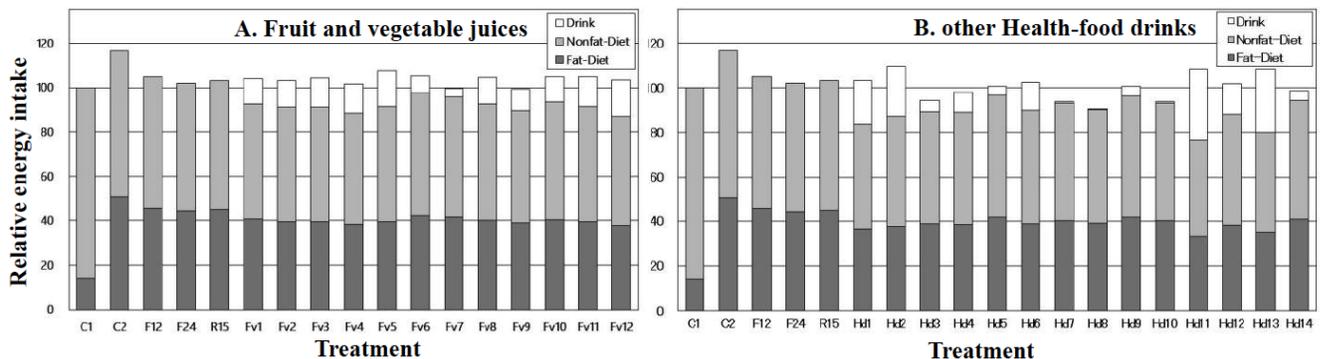
Figure 4: Relative total energy intakes from fat- and non-fat-components in the diet and from drinks for mice of the control groups and the experimental drink groups exposed to restraint repeatedly for 15 min per day under concomitant administration of high-fat diet and one of twelve fruit and vegetable juices and the other fourteen health-food drinks.

C1, standard diet control; C2, high-fat diet control; F12 and F24, high-fat diet plus fasting for 12 h and 24 h, respectively; R15, high-fat diet plus restraint for 15 min. Mice were exposed to stress daily on weekdays in weeks 2 to 4 of the 4-week study period, except for F24 (every other day) mice. Data of the control groups (C1, C2, F12, F24 and R15) and the experimental drink groups are presented as mean obtained from two experimental groups for twelve fruit and vegetable juice groups (A. Fv1-12) and for the other fourteen health-food drink groups (B. Hd1-14), n=2.
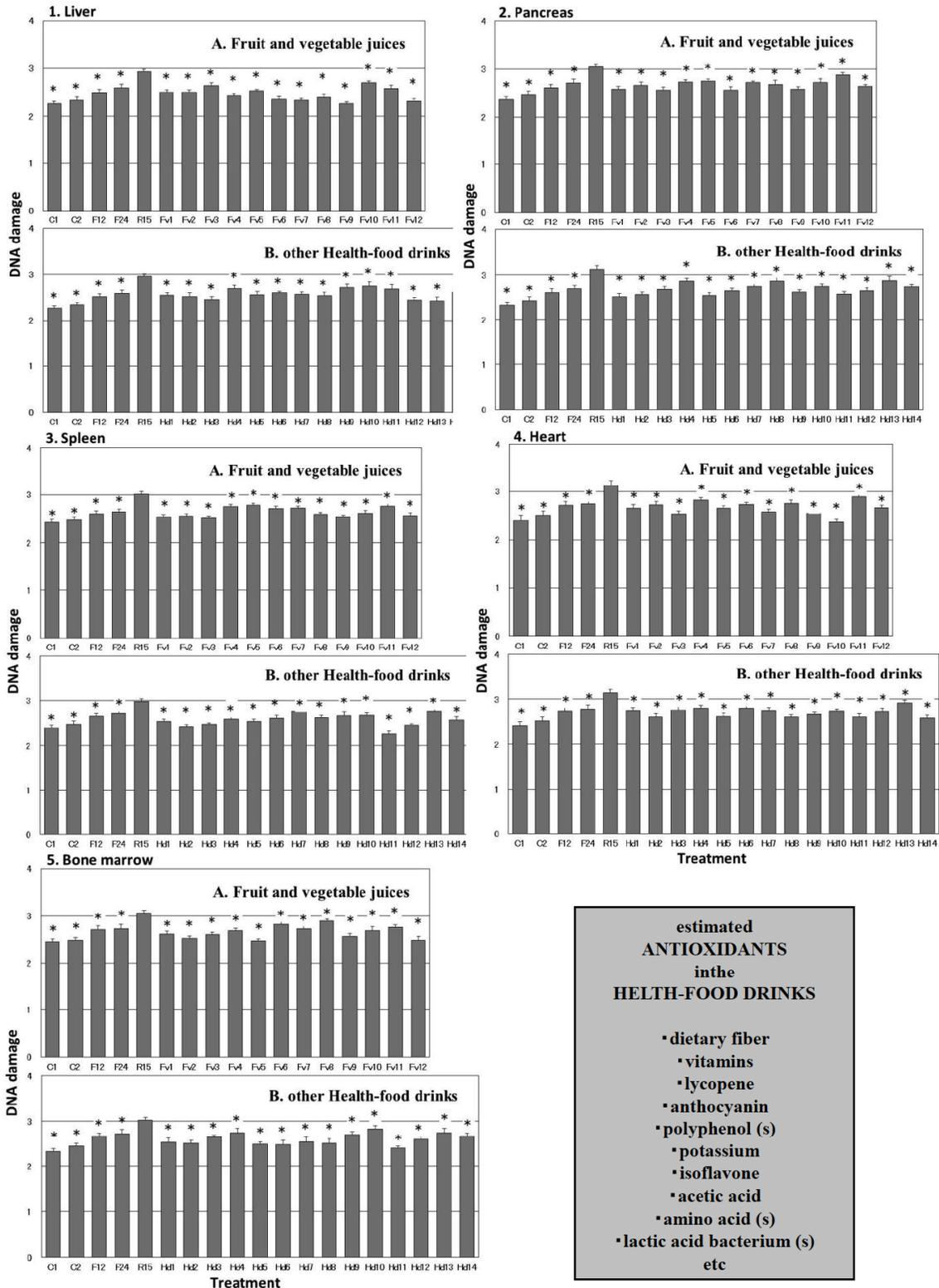
Figure 5: DNA damages in the (1) liver, (2) pancreas, (3) spleen, (4) heart and (5) bone marrow of the control groups, and the experimental drink groups exposed to restraint repeatedly for 15 min per day under concomitant administration of high-fat diet and one of twelve fruit and vegetable juices and the other fourteen health-food drinks.

C1, standard diet control; C2, high-fat diet control; F12 and F24, high-fat diet plus fasting for 12 h and 24 h, respectively; R15, high-fat diet plus restraint for 15 min. Mice were exposed to stress daily on weekdays in weeks 2 to 4 of the 4-week study period, except for F24 (every other day) mice. Data of the control groups (C1, C2, F12, F24 and R15) and the experimental drink groups for twelve fruit and vegetable juice groups (A. Fv1-12) and for the other fourteen health-food drink groups (B. Hd1-14) are presented as mean ± SEM obtained from each 100 cells. * $p < 0.05$ vs. corresponding R15 group.

groups were generally reduced a little compared with those of the corresponding restraint control groups (R15), the reduced energy was compensated by the energy from the fruit and vegetable juice. The total energy intake in some of the other health-food drink groups was a little lower than that of the restraint control group (R15) because of the low energy content of the health-food drinks, which may contain artificial sweetener(s). In general, the fat-energy intakes in all HFD groups were much higher than that in the STD control group (C1).

DNA Damage

DNA damage in the liver, pancreas, spleen, heart and bone marrow of control groups and experimental drink groups exposed to restraint repeatedly for 15 min per day under concomitant administration of high-fat diet and one of twelve fruit and vegetable juices or the other fourteen health-food drinks is shown in Figure **5**. There were significant differences between the R15 group and all treatment groups.

DISCUSSION

Childhood obesity, which is associated with metabolic syndrome, diabetes, heart disease and cancer, is one of the most debilitating and serious public health issues worldwide [4, 8, 9]. Diabetes is among the most serious morbidities resulting from childhood obesity and its prevalence is now increasing globally. One in eleven adults in the world has diabetes according to the estimation by the International Diabetes Federation (IDF) [22]. Diabetes and its complications are major causes of death in most countries and the increase in prevalence of these diseases is seen not only in adults but also in child and adolescents. Diabetes is classified into type 1 and type 2 and approximately 87% to 91% of people with diabetes are estimated to have type 2, which is a typical lifestyle-related disease. Healthy eating habits and increased physical activity are highly recommended for preventing the risk of type 2 diabetes [22, 23].

In the present study, we documented that body weight gain in the mice was considerably reduced by repeated restraint for 15 minutes per day (Figures **1** and **2**) and that the reduction of body weight gain was further promoted by concomitant administration of fruit and vegetable juices and other health-food drinks, with a decrease in abdominal fat weight (Figure **3**). While some benefits of stress including suppression of body weight gain were observed, mild DNA damage was seen in the cells of murine organs as previously

described [15], which was clearly suppressed by the concomitant administration of the health-food drinks (Figure **5**). Both the decrease in body weight gain and the suppression of DNA damage may be due to the additive action and/or synergistic effects of the antioxidants including dietary fiber, vitamins, lycopene, anthocyanin, polyphenol, potassium, isoflavone, acetic acid, amino acid(s), and lactic acid bacteria, and so on [2, 17, 18]. It has been shown that mental and physiological stresses induce excess reactive oxygen species (ROS) and worsen lifestyle-related diseases [3, 5, 6]. The presence of excess ROS can cause cellular damage via oxidation of biomolecules, including DNA, proteins and polyunsaturated fatty acids, causing a variety of diseases such as cancer, Parkinson's disease, and cardiovascular disease [1, 2]. To reduce the formation of excess ROS is important for preventing the development of diseases.

Antioxidants are compounds abundant in fresh fruits and vegetables [2]. A number of publications have demonstrated that adequate consumption of plant foods is associated with decreased risk of chronic degenerative diseases, such as coronary heart disease, stroke, diabetes or certain types of cancer, whose risk-reducing effect are attributed to the components including phytochemicals, phytonutrients and several vitamins, minerals and fiber [24]. While many epidemiological studies have consistently shown that regular consumption of fruits and vegetables is strongly associated with reduced risk for developing chronic diseases, the actions of antioxidant nutrients alone do not explain the observed health effects of diet rich in fruits and vegetables, because, if taken alone, the individual antioxidants studied in clinical trials do not appear to have consistent preventive effects [25]. The development of synergistic effects of free radical scavengers in fruits and vegetables needs to be further investigated, and several pilot studies with vitamins such as ascorbic acid, tocopherol and β-carotene are currently underway [18, 26, 27]. We described here the suppression of DNA damage generated by mild stress in the cells of murine organs by the use of concomitant administration of health-food drinks mixed with fruits and vegetables commercially available.

Based on the results of this study, it was shown that DNA damage of the cells in multiple murine organs could be suppressed by the combination of health-food drinks and mild stress exposure, suggesting that some antioxidative foods may be able to prevent the development of lifestyle-related diseases associated with obesity and hyperlipidemia in combination with

beneficial effects of mild stress [7, 16], not only in the experimental animals described here but also in humans.

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