ORIGINAL

Awa (Tokushima) lactate-fermented tea as well as green tea enhance the effect of diet restriction on obesity in rats

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Abstract : Drinking tea is recommended for promoting health due to its bioactive nutrients, such as catechins and caffeine. In Tokushima area, we have a unique traditional tea, named Awa tea, which are fermented with *Lactobacillus pentosus and Lactobacillus plantarum*. The present study was designed to investigate anti-obesity effects of the Awa tea and compare with those of non-fermented green tea. Obese male Wistar rats (19 weeks of age) were given by low energy diets containing 3% of Awa and green tea extracts, respectively, or without any tea extracts (control), for 4 weeks. Awa tea contained smaller amount of catechins than green tea, although they contained similar amounts of polyphenols. This finding indicates that there are distinct kinds of polyphenols from catechins. The diets containing Awa and green tea extracts further decreased whole body weight, fat tissue mass and plasma leptin level, compared with control diet. In addition, their diets increased the daily amount of lipid excreted to feces and total 24-h-energy consumption, compared with the control group. However, there is no significant difference in these anti-obesity effects between Awa tea and green tea. Our results indicate that Awa lactate-fermented tea as well as green tea similarly enhance the effect of diet restriction on obesity, at least in part, through the increase in fat energy consumption and the decrease in fat absorption in rats. J. Med. Invest. 56 : 42-48, February, 2009

Keywords **:** *Awa (Tokushima) tea, high-fat diet, energy restriction, anti-obesity, rats*

Abbreviations :
AIN, American Institute of nutrition ; ANOVA, analysis of variance ; ELISA, enzyme-linked immunosorbent assay ; HPLC, high performance liquid column chromatography.

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INTRODUCTION

Obesity is a serious and increasing public health challenge in the developed countries including Japan, because it is a major risk factor for metabolic syndrome, such as diabetes mellitus type 2, cardiovascular diseases and several types of cancer (1, 2). In Japan, it is estimated that approximately 13 million of adults have obesity, with the prevalence approaching 50% in middle-aged and elder men. Obesity results from disequilibrium between energy intake and expenditure. However, it is difficult to decrease energy intake and/or increase energy expenditure without dietician's help.

Catechins are one group of the most bioactive polyphenols in green tea, which is a popular beverage consumed with foods in Japan. Catechins have a potent anti-obesity action, because they reduced plasma fatty acids and cholesterol concentrations (3, 4) and prevented hepatic and body fat accumulation (5-9). Therefore, dietitians generally recommend drinking green tea in lifestyle to primarily prevent and improve obesity-associated diseases.

In Tokushima (Awa), we have a unique traditional tea, which consists of leaves fermented with *Lactobacillus pentosus and Lactobacillus plantarum*. Fermentation may modify the structure of bioactive substances, such as polymerization of catechins and formation of novel polyphenols, resulting in the enhancement of bioactive functions, as well as improves taste. In fact, the lactic acid fermentation normalizes enterobacterial balance (10, 11) and increases the ability scavenging superoxide anions (12, 13). Based on these findings, the present study was designed to examine whether Awa (Tokushima) lactate-fermented tea had anti-obesity actions compared with normal green tea.

MATERIALS AND METHODS

Animals and diets

Male Wistar rats (8-week-old of age) purchased from Charles River Laboratories Japan (Atsugi, Tokyo) were individually housed in a room controlled at 23±2°C and 50% humidity with a 12 h-light-dark cycle. The animal experiment in this study consisted of the following two phases : weight-gain and weight-loss phases. In a weight-gain phase, all rats were free to access to water and a high-fat diet, in which 26% lard was added to a standard stock diet (Oriental Yeast, Osaka, Japan), for 12 weeks. Then, they were subjected to a weight-loss phase for 4 weeks. In a weight-loss phase, rats were randomly divided to three groups and fed three kinds of experimental diets, 20% casein diet without excess fat (control diet group), 20% casein diet supplemented with either 3% green tea extract (green tea group) or 3% Awa tea extract (Awa tea group), respectively. These tea extracts are prepared by Maruzen Pharmaceuticals (Hiroshima, Japan). Detail compositions of these diets were listed in Table 1. During a weight-loss phase, the amounts of all tested diets were restricted to 11.9 g, which is corresponding to about 50% of food intake of the same-aged rats. All feces were collected for three days in the weight-loss phase and dried by heating at 105°C for 24 h. On the last day, the blood was collected from vena cava under anesthesia with pentobarbital sodium (1 mg/kg body weight, Nembutal™, Dainippon Pharmaceutical, Osaka, Japan) after overnight fasting. Isolated tissues, such as liver, stomach, gastrocnemius muscle and adipose tissue, were weighed. The carcasses including tissues were snap-frozen with liquid nitrogen and kept at -80°C until analysis.

Table 1. Composition of experimental diets.

Diet	Control	Green tea[3]	Awa tea[3]
			(g/100 g diet)
Casein[1]	20.0	20.0	20.0
α-Corn starch	45.7	43.7	43.7
Sucrose	22.8	21.8	21.8
Corn oil	5.0	5.0	5.0
Vitamin mixture[2]	1.0	1.0	1.0
Mineral mixture[2]	3.5	3.5	3.5
Cellulose	2.0	2.0	2.0
Tea extract[3]	0	3.0	3.0

[1]Casein was purchased from Oriental Yeast (Osaka, Japan).
[2]AIN (American Institute of Nutrition)-93 (30).
[3]Dry leaves of green or Awa teas were extracted with boiled water, and the extracts were evaporated.

All protocols described in the present study were conducted according to the Guide for the Care and Use of Laboratory Animals at Shikoku University and were approved by the Committee of the Care and Use for Laboratory animals at Shikoku University.

Energy consumption

Energy consumption for 24 h was measured by the indirect calorimetry (14) at the middle time point during a weight-loss phase. Briefly, rats were placed for 24 h in the respiratory chamber, which was sufficiently airtight to control the flow rate (at about 3 L/min) and monitored the concentrations of oxygen and carbon dioxide. The oxygen consumption (V_{O2}) and the carbon dioxide discharge (V_{CO2}) of rats were calculated by a metabolism measurement system for small animals (Muromachi Kikai Co., Tokyo, Japan).

Body composition in rats

Carcasses were dried by heating at 105°C for 24 h, followed by homogenization with a mixer. Fat was extracted from the homogenized carcasses with ether, using a Soxhlet apparatus (15). The fat ratio of the carcass was ascertained by measuring the weight of extracted lipid. Other body compositions in rats were calculated from the values of whole wet weights, lean body mass and fat mass.

Other biochemical analysis

Plasma insulin and leptin concentrations were determined by the respective enzyme-linked immunosorbent assay (ELISA) kits (Shibayagi, Gunma, and Wako, Osaka, Japan). Lipids were extracted from dried feces or teas by the procedure of Folch, *et al.* (16), and their amounts were gravimetrically determined. Amounts of other nutritional compositions, such as protein, minerals and polyphenols, in green and Awa tea extracts were measured by the respective standard methods. Briefly, protein contents were calculated by using nitrogen-protein conversion factor (nitrogen×6.25), and the amounts of nitrogen were measured by the Kjeldahl method (17). The amounts of carbohydrate were determined by the Somogyi-Nelson method (18). Ash contents were ascertained by measuring the weight of the sample after heated at 550°C for an appropriate period. Water contents were ascertained by measuring wet and dry weights. Dietary fibers were measured by the method of Prosky, *et al.* (19). The amounts of polyphenols, and levels of catechins and caffeine were quantified by the methods of Singleton, *et al.* (20) and Saijo, *et al.* (21), respectively, using high performance liquid column chromatography (HPLC).

Statistical analysis

All values are presented as mean±SD. Statistical comparisons of the groups were performed by analysis of variance (ANOVA), and each group was compared with the others by Tukey's honestly significant difference test (JMP, SAS Institute Inc., NC, USA). Differences were considered significant at $P < 0.05$.

RESULTS

Compositions of green and Awa tea extracts

To address whether lactate-fermentation changes the contents of green tea, we examined compositions of green and Awa tea extracts (Table 2). All compositions of green and Awa tea extracts were similar, except for amounts of carbohydrate and catechins. The amount of carbohydrate was decreased, because fermentation requires glucose. The amount of catechins was decreased in Awa tea extract, compared with green tea extract, although amounts of total polyphenols in both tea extracts were similar.

Table 2. Composition of green and Awa tea extracts.

	Green tea	Awa tea
		(%)
Carbohydrate	20.8	11.8
Protein	17.3	21.3
Lipid	0.9	0.4
Ash	6.2	7.8
Water	4.1	6.7
Dietary fiber	4.2	4.9
Polyphenols	29.0	29.6
Catechins	20.0	8.4
Caffeine	5.0	7.6
Others	12.5	9.9

Compositions of tea extracts were analyzed by the respective standard methods as described in MATERIALS AND METHODS. Polyphenols are chemicals, which possess more than two phenol stuructures. Catechins are categorized to polyphenol group, because they have two or three phenols.

Effects of green and Awa teas on body weight loss and body fat ratio

In a weight-gain phase, the *ad libitum* feeding of a high fat diet for 12 weeks increased body weight and body fat ratio in rats to about 531±50 g and 24.7±6.1%, respectively. In a weight-loss phase, the restricted feeding of a control diet for 4 weeks did not affect body fat ratio, whereas it significantly decreased body weight in rats (Fig. 1, A and B). Diets supplemented with green or Awa tea extract decreased the body weight in rats more than a control diet. Furthermore, these diets decreased the

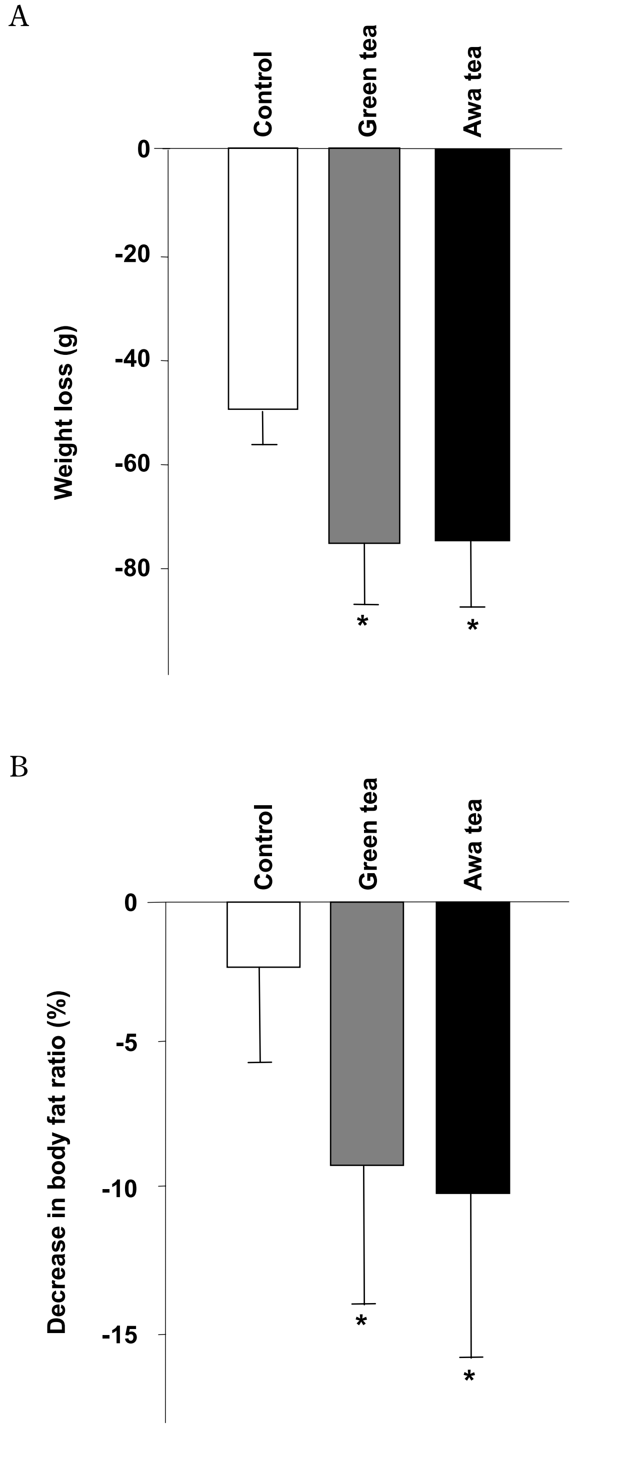

Fig. 1. Body weight loss and decrease in body fat ratio in rats fed experimental diets.
In a weight-loss phase, obese rats fed a control diet, or diets containing green or Awa tea extract for 4 weeks. After the weight-loss phase, body weight loss (A) and body fat ratio (B) were measured. Values are expressed as mean ± SD (n=6). *Significantly different from control group, $P < 0.05$.

body fat ratio, compared with that in the control diet group. However, there were no significant differences in the body weight and the body fat ratio between the green tea and Awa tea.

Effects of green and Awa teas on visceral fat amounts

Wet weights of liver, kidney, stomach and gastrocnemius muscle in rats fed three kinds of diets were not different (data not shown). We found that diets supplemented with green and Awa tea extracts significantly reduced visceral fat accumulation, when we examined the distribution of fat in three individual fat pads, i.e., epididymal, perinephric and mesenteric adipose tissues (Table 3). The supplementations with green and Awa tea extracts significantly reduced wet weights of epididymal and perinephric adipose tissues, compared with those in the control group, although they did not change weight of mesenteric adipose tissue. These results suggested that feeding of an Awa tea diet as well as green tea diet is effective for the suppression of body fat accumulation *in vivo*.

Table 3. Effects of experimental diets on visceral fat weight.

	Dietary group		
	Control	Green tea	Awa tea
			(g)
Epididymal	14.57 ± 3.09	8.62 ± 2.56*	7.85 ± 3.67*
Mesenteric	5.47 ± 2.42	3.43 ± 2.40	2.35 ± 1.66
Perinephric	16.03 ± 3.43	7.76 ± 4.08*	6.48 ± 2.58*

Obese rats were subjected to a weight-loss phase for 4 weeks. During this phase, obese rats fed a control diet, or diets containing of green or Awa tea extracts. On the last day, visceral fats were isolated and weighed. Values are expressed as mean ± SD (n=6). *Significantly different from control group, $P < 0.05$.

Lipid absorption and energy consumption of rats fed green tea and Awa tea diets

To address the mechanism of these effects on obesity, we measured total amounts of ingested and fecal lipids for 3 days and total 24-h-energy consumption. Total amounts of fecal lipids in rats fed green and Awa tea diets were larger than that in rats fed a control diet, whereas total amount of ingested lipids in three diet groups were constant (Table 4). This finding suggested that diets containing green and Awa tea extracts significantly inhibited the lipid absorption rate, compared with a control diet. Total 24-h-energy consumption in Awa tea diet group was also higher than that in control diet group (Fig. 2). The green tea diet group also showed the tendency of higher consumption than the control group, however, the difference was not statistically significant.

Table 4. Effects of experimental diets on lipid absorption.

	Dietary group		
	Control	Green tea	Awa tea
Total amounts of ingested lipids (g)	1.80	1.80	1.80
Total amounts of fecal lipids (g)	0.13± 0.03	0.25± 0.10*	0.25± 0.07*
Lipid absorption rate (%)	92.75± 1.59	86.23± 5.25*	86.50± 3.80*

Obese rats were subjected to a weight-loss phase for 4 weeks. During this phase, obese rats fed a control diet, or diets containing of green or Awa tea extracts. Feces were collected for three days in the weight-loss phase, and total amounts of lipids in all faces were measured. Lipid absorption rate was calculated by the following quotation : (total amounts of ingested lipids-total amounts of fecal lipids)/total amounts of ingested lipids. Values are expressed as mean± SD (n=6). *Significantly different from control group, $P<0.05$.

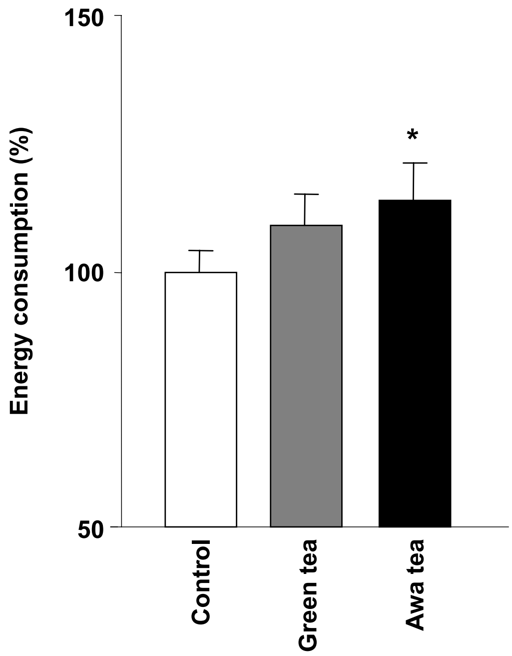

Fig. 2. Effects of experimental diets on total 24-h-energy consumption in rats.
In a weight-loss phase, obese rats fed a control diet, or diets containing green or Awa tea extracts for 4 weeks. At the middle time point during a weight-loss phase, total 24-h-energy consumption of rats was measured by the indirect calorimetry. Values are expressed as mean± SD (n=6). *Significantly different from control group, $P<0.05$.

Plasma insulin and leptin levels of rats fed green tea and Awa tea diets

Since plasma insulin and leptin levels are associated with body weight and fat ratio in animals and human beings (22, 23), we also measured these parameters of rats in all tested diet groups. Plasma insulin levels are not significantly different among all diet groups (Fig. 3A). In contrast, diets supplemented with green and Awa tea extracts decreased plasma leptin concentration, compared with a control diet (Fig. 3B).

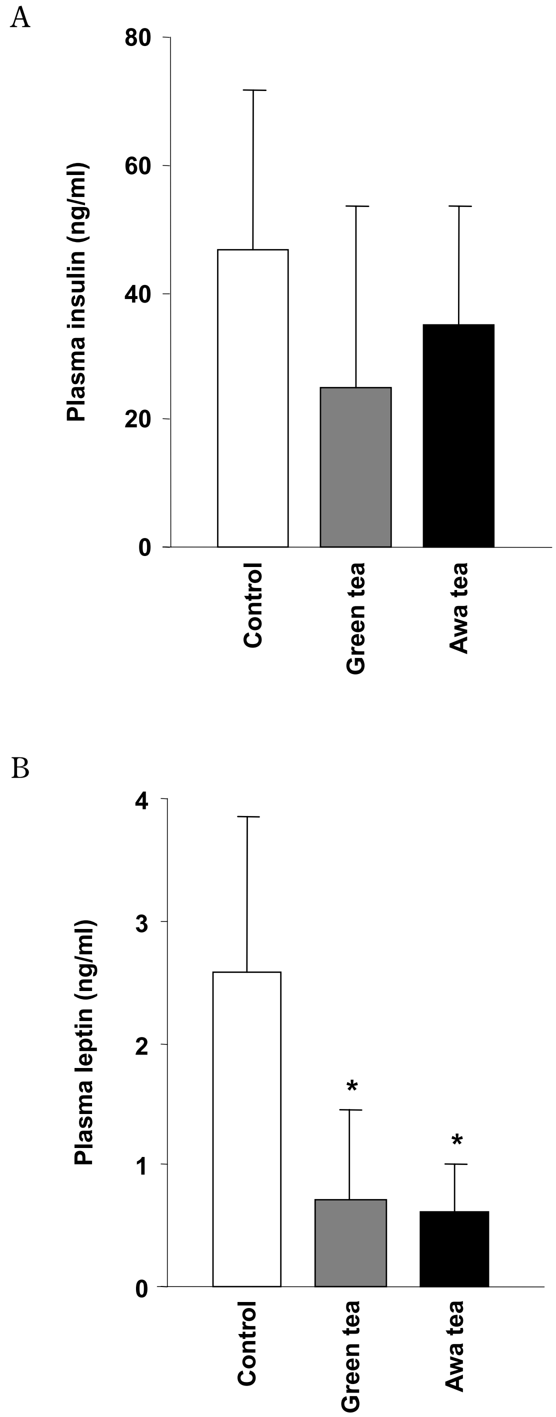

Fig. 3. Plasma insulin and leptin concentrations in rats fed experimental diets.
Obese rats were subjected to a weight-loss phase for 4 weeks. During this phase, obese rats fed a control diet, or diets containing green or Awa tea extract. On the last day, blood was collected, and plasma insulin (A) and leptin (B) concentration were measured with the respective ELISA. Values are expressed as mean± SD (n=6). *Significantly different from control group, $P<0.05$.

DISCUSSION

In Tokushima (Awa), we have a unique traditional tea, which consists of leaves fermented with *Lactobacillus pentosus and Lactobacillus plantarum*. Since fermentation could occasionally produce bioactive substances in tea, we examined inhibitory effects of Awa tea on obesity and compared with those of non-fermented green tea. The present results showed that Awa tea extract had similar anti-obesity effects as green tea extract : they significantly enhanced diet restriction-mediated decreases in body fat ratio and visceral fat weight. Green tea is well known as a representative anti-obesity food, since it prevents lipid absorption (24, 25) and stimulates energy consumption (26, 27). In the present study, we showed that Awa tea improved lipid metabolism. However, there was no difference between Awa tea and green tea.

The preparation processes between green tea and Awa tea are very different. During green tea preparation, there is a process heating leaves for 30-60 s, resulting in inactivation of enzymes (28). In contrast, leaves of Awa tea were similarly heated, followed by the fermentation with lactic acid bacteria, accompanied with the activation of various enzymes, such as polyphenoloxidase (28, 29). Therefore, we expected that these bacteria could produce novel bioactive substances in Awa tea. In fact, we found that the amount of catechins was decreased in Awa tea, compared with green tea, although the amounts of total polyphenols in both teas were similar. This finding suggests that lactate-fermentation metabolizes catechins to other polyphenols.

Catechins mainly contribute to anti-obesity actions of green tea. A lot of investigations demonstrated that catechins prevented lipid absorption (24, 25). In addition, catechins increased fat oxidation and energy expenditure in human and rat brown adipose tissue, possibly by stimulating sympathetic nerve system (26). In Awa tea extract, the amount of catechins was small, compared with that of green tea extract. Nevertheless, Awa tea had similar anti-obesity actions. It is likely that polyphenols produced by fermentation have same actions as catechins. Further examinations are necessary to identify the bioactive polyphenols in Awa tea.

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