香川大学農学部学術報告　第75巻　63～71，2023

Dietary Green Tea Powder Supplementation Reduces Body Fat Accumulation and Improves Insulin Resistance in Rats

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Abstract

Obesity is an important public health issue and a major cause of global health problems. Green tea is highly valued for its health benefits against obesity. However, most previous studies have used green tea extract as the experimental material and studies using green tea powder are limited. In this study, we examined the effects of dietary green tea powder supplementation on body fat accumulation and insulin resistance in rats that were fed a high-fat and high-sucrose diet, one that typically causes obesity. Male Wistar rats（3-weeks-old）were fed either a high-fat and high-sucrose diet（C diet）or a 5% green tea powder-supplemented diet（G diet）for eight weeks. Final body weight, weight gain, food intake, food efficiency, and heart and kidney weights did not differ between the groups. However, the weights of intra-abdominal adipose tissues, carcass fat, and total body fat were significantly lower in Group G than that in Group C. Homeostatic model assessment of insulin resistance（HOMA-IR）was significantly lower, and the quantitative insulin sensitivity check index（QUICKI）was higher in Group G compared with Group C. These results suggested that dietary green tea powder supplementation significantly reduced body fat accumulation in rats and improved insulin resistance. Green tea powder has anti-obesity effects and can be used as excellent functional food.

Key words : Green tea powder, body fat, insulin resistance, rat

Introduction

Obesity is an important public health problem and a major global health concern[(1)]. Obesity significantly increases the risk of non-communicable diseases such as diabetes mellitus, cardiovascular disease, and cancer[(2)]. According to the Japan Society for the Study of Obesity（JASSO）, obesity is characterized by abnormal or excessive fat accumulation and is clinically diagnosed based on a body mass index（BMI）$>$ 25（kg/m^2）[(3)]. In 2019, the percentage of obese Japanese people aged $\geq$ 20 years was 33.0% for men and 22.3% for women, and is increasing yearly[(4)]. Obesity is a multifactorial chronic disease that results from an imbalance between energy intake and expenditure[(5)]. Dietary and lifestyle changes are the main factors recommended in the treatment of obesity[(6)]. Moreover, the WHO Global Strategy on Diet, Physical Activity, and Health promotes physical exercise and healthy eating[(7)]. While the importance of diet and physical activity is well known, the use of supportive herbal remedies may also become part of the treatment of obesity in the coming years[(8)]. Green tea supplementation may be among these, which has been intensively studied over the last decade[(9)].

Green tea is a major type of tea（Camellia sinensis）and belongs to the non-fermented tea class[(10)]. Of all tea types, the most important impact on human health has been observed in green tea consumption[(9)]. Green tea is highly valued for its health benefits against obesity, cancer, osteoarthritis, hypercholesterolemia, hyperglycemia, and neurodegenerative diseases[(11)]. The health benefits of green tea are likely related to the antioxidant properties associated with its high content of GT polyphenols（including catechins and flavonols）[(12)].

The effects of green tea on obesity have been discussed in several studies. In human studies, green tea had anti-obesity effects by inhibiting ghrelin secretion, increasing adiponectin levels, substrate oxidation, appetite control, reducing nutrient absorption, and inhibiting fat production[(9, 13)]. In animal studies, anti-obesity mechanisms may have been based on the activation of brown adipose tissue, decreased food intake, inhi-

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bition of fatty acid absorption, alteration of the microbiota, and increase in hepatic adenosine monophosphate-activated protein kinase（AMPK）phosphorylation[14-18]. However, most of these studies used green tea extract as the experimental material while studies using green tea powder have been limited. In Japan, there is a tradition of drinking a whisked green tea powder called “Sado”（tea ceremony）, and there are many foods containing green tea powder, including soft drinks, cakes, and ice cream.

In this study, we examined the effects of dietary green tea powder supplementation on body fat accumulation and insulin resistance in rats fed a high-fat and high-sucrose diet that typically causes obesity, to reconfirm the effects of green tea powder.

Materials and Methods

All animal procedures were approved by the Animal Care and Use Committee for Kagawa University（approval number：17625）.

Materials

Green tea powder was procured from Yamashiro Bussan Co., Ltd.（Osaka, Japan）. The composition of the components in 100 g of dry green tea powder was 24.5 g of protein, 4.7 g of fat, 23.0 g of non-fiber carbohydrate, 48.5 g of dietary fiber, 5.0 g of ash, and 2.8 g of water. Vitamin and mineral mixtures（AIN-76A）were procured from Oriental Yeast Co. Ltd.（Tokyo, Japan）. Soybean oil with the following composition was procured from Yamakei Industry Co. Ltd.（Osaka, Japan）：52.7% linoleic acid, 24.3% oleic acid, 7.9% α-linolenic acid, 10.3% palmitic acid, and 3.8% stearic acid.

Animals and experimental design

Sixteen male Wistar rats（3-weeks-old）were procured from Japan SLC（Shizuoka, Japan）and randomized into two groups of eight rats. They were individually caged at 22 ± 1°C, under light from 08：00 h to 20：00 h. They were fed MF, a commercial rodent diet（Oriental Yeast Co., Ltd., Tokyo, Japan）, and had access to water *ad libitum* for four days. The initial body weight of the rats was 64.8 ± 0.8 g（range：61.5–69.0 g）. The rats were then fed a high-fat and high-sucrose diet（C diet）or supplemented with a 5.0% green tea powder diet（G diet）（Table 1）with free access to water for eight weeks. The two diets contained 17.6% protein, 25.4% fat, and 43.0% non-fibrous carbohydrates. The C and G diets contained 5.0% and 4.7% of dietary fiber, respectively. Body weights were recorded daily. After the experimental period, all rats were euthanized by beheading at 09：00 h. Blood was collected to obtain serum. Heart, liver, kidneys, spleen, and abdominal adipose tissues（epididymal, perirenal, and mesenteric）were quickly removed and stored at −80°C until analysis. Carcass samples were obtained by removing the head and remaining intra-abdominal and intra-thoracic tissues. They were stored at −20°C until carcass fat analysis was conducted.

Table 1 Composition of experimental diets（g/kg）.

Ingredients	C diet	G diet
Casein	200.0	189.6
DL-Methionin	3.0	3.0
Corn starch	249.9	249.9
Sucrose	200.0	189.2
Green tea powder	0.0	50.0
Soybean oil	50.0	48.2
Beef tallow	200.0	200.0
Mineral mixture[1]	35.0	35.0
Vitamin mixture[1]	10.0	10.0
Cellulose	50.0	23.0
Chorine chloride	2.0	2.0
Butylhydroxytoluene	0.1	0.1

[1]Based on the AIN76 mixture.
All diets contain 175.6g/kg of protein, 254.3g/kg of fat, and 429.6g/kg of non-fiber carbohydrates.

Serum and liver analyses

The concentrations of serum glucose, insulin, total cholesterol, HDL-cholesterol, free fatty acids（FFA）, and triglycerides were determined using kits（Glucose C Ⅱ − test, LBIS Rat Insulin ELISA Kit, Cholesterol E-Test, HDL-Cholesterol E-Test, NEFA C-Test, and Triglyceride E-Test［FUJIFILM Wako Pure Chemical Corporation, Osaka, Japan］）. The homeostatic model assessment of insulin resistance（HOMA-IR）was calculated using the method described by Matthews et al.[19]. Quantitative insulin sensitivity check index（QUICKI）was calculated using the method described by Katz et al.[20].

Liver lipids were extracted using the method described by Folch et al.[21], and the liver triglyceride and cholesterol contents were determined using kits（Triglyceride E-Test and Cholesterol E-Test, ［FUJIFILM Wako Pure Chemical Corporation］）. Liver glycogen content was determined using the method described by Lo et al.[22].

Carcass analyses

Carcass fat content was analyzed using the method described by Mickelsen and Anderson[23]. Briefly, fat from auto-

claved, homogenized, and dried carcass samples were extracted using a Soxhlet extractor. Carcass fat percentage was determined as the ratio of carcass fat mass to carcass weight. Total body fat was calculated using the method described by Paik and Yearick[24]. The total fat mass was the sum of carcass fat mass and intra-abdominal fat mass multiplied by 0.85. The total fat percentage was calculated as the ratio of total fat mass to the final body weight. The feces were dried, and lipids were extracted using the Soxhlet method.

Data analysis

All data were analyzed using an unpaired Student's t-test. Statistical significance was set at $p < 0.05$. The correlation of intra-abdominal adipose tissue weight, carcass fat mass, and total fat mass with HOMA-IR and QUICKI were calculated using Pearson product-moment correlation coefficients.

Results and Discussion

Fig. 1 shows the body and tissue weights, food intake, and food efficiency. Final body weights, weight gains, food intake, food efficiency, and heart and kidney weights did not differ between the groups. Liver and spleen weights were significantly lower in Group G than in Group C. Fig. 2 shows the intra-abdominal adipose tissue weights, carcass fat, and total body fat. The amount of intra-abdominal adipose tissues (epididymal, perirenal, mesenteric, and total adipose tissues), carcass fat (weight and percentage), and total body fat (weight and percentage) were significantly lower in Group G than in Group C. These results suggested that green tea powder had a strong anti-obesity effect supporting many previous studies on green tea extract or green tea polyphenols[25–29]. Zhang et al.[29] indicated that polyphenols and polysaccharides were responsible for the suppressive effect of green tea extracts on body weight increase and fat accumulation in rats. Moreover, Rasso, et al.[27] demonstrated that chronic consumption of green tea extract leads to a decreased visceral adipose tissue. Many studies have reported that the anti-obesity effect of green tea extract is due to polyphenols, especially catechins. Green tea powder contains approximately 15% catechins, most of which are epigallocatechin and epigallocatechin gallate (approximately 4% and 8%, respectively)[30]. In the present study, it was estimated that the G diet contained approximately 0.75% tea catechins. The intake of catechins in Group G was approximately 10 mg/day, which is the same level as that reported in

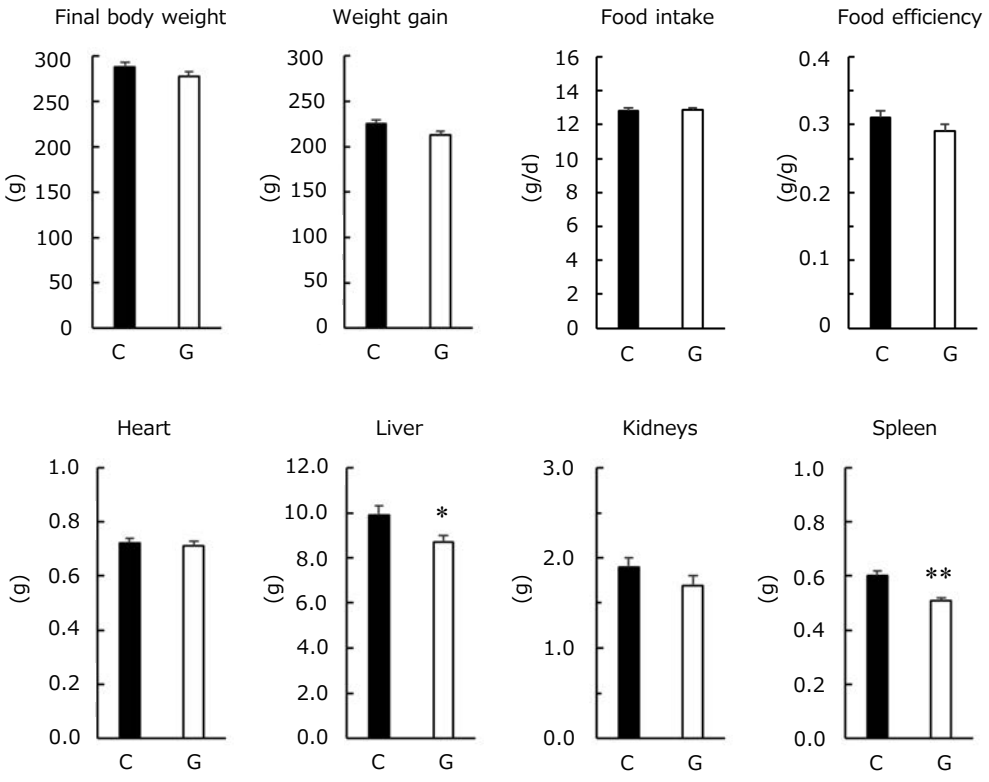

Fig. 1 Final body weight, weight gain, food intake, food efficiency, and tissue weights in rats fed the control (C) diet and the green tea powder supplemented diet (G). Values are means ± SE for 8 rats. $^{**}p < 0.01$, $^{*}p < 0.05$ vs. group C (Student's t-test).

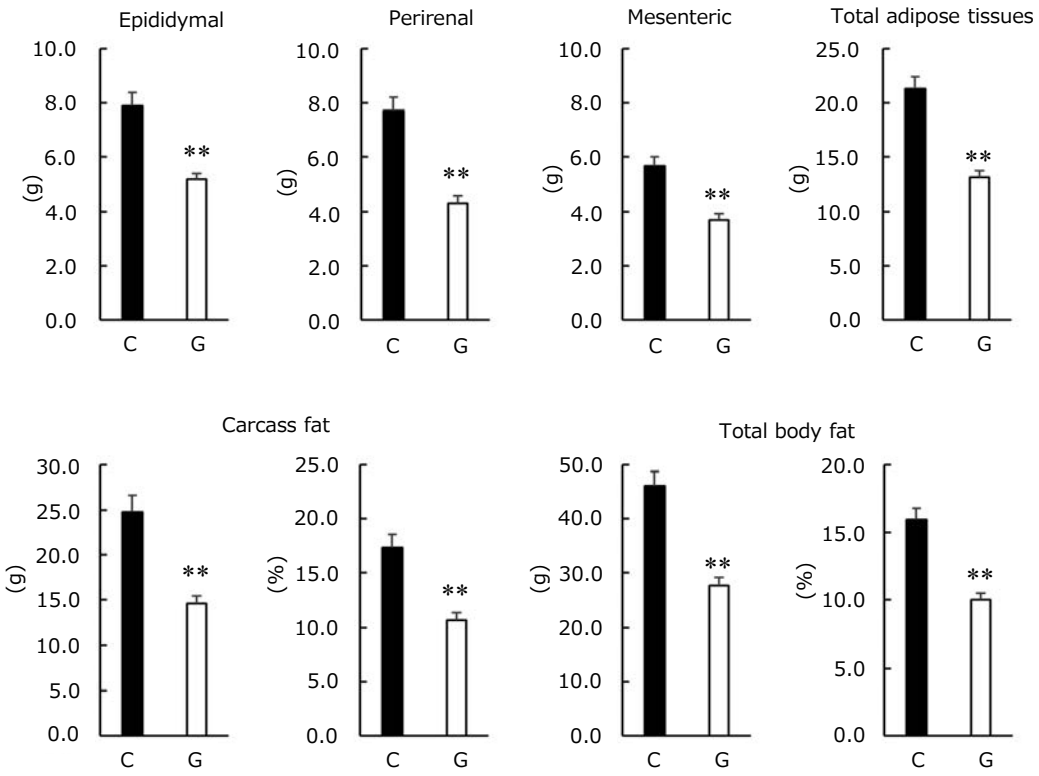

Fig. 2 Intra-adipose tissue weights, carcass fat, and total body fat (weights and percentages) in rats fed the control (C) diet and the green tea powder supplemented diet (G). Values are means ± SE for 8 rats. **p <0.01 vs. group C (Student's t-test).

previous studies using green tea extract [25-29]. Thus, the anti-obesity effect of green tea powder may be due to the catechins present in green tea.

Figs. 3 and 4 show the results of the serum and liver biochemical tests, respectively. Serum insulin, total cholesterol, HDL-cholesterol, and triglyceride concentrations were significantly lower in Group G than in Group C, whereas serum glucose concentration did not differ between Groups C and G. There was no difference in the triglyceride and glycogen contents per liver between Groups C and G, whereas the total lipid and cholesterol contents per liver were significantly lower in Group G than in Group C, suggesting that green tea powder supplements have lipid-reducing effects on the serum and liver. Our study, at least in part, supports previous reports on the serum and liver lipid reducing effects of green tea extract and tea catechins [31-33]. In contrast, serum FFA concentration and HOMA-IR were significantly lower and QUICKI was higher in Group G compared with Group C (Fig. 4). Serum FFA may be an intermediary factor in impaired insulin sensitivity and glucose tolerance related to obesity and type-2 diabetes mellitus [34-36]. A decrease in circulating FFA and fatty acid oxidation is expected to improve hyperglycemia and strengthen the insulin response by suppressing glucose production and increasing glucose utilization [37]. The results of this study demonstrated that dietary green tea powder supplementation improved insulin resistance.

However, systematic reviews and meta-analyses investigating the relationship between green tea consumption, insulin resistance and glycemic control have reported mixed findings. One meta-analysis concluded that green tea consumption was effective in decreasing fasting glucose and glycemic control (HbA1c concentration) in both healthy subjects and patients with obesity or metabolic syndrome [38]. In contrast, Wang et al. [39] found no effect of green tea consumption on fasting glucose, insulin, glycemic control, or insulin resistance in participants with type-2 diabetes mellitus. Additionally, Yu et al. [40] performed a meta-analysis to assess the biomarkers of insulin resistance and glycemic control between green tea and placebo groups. Overall, no differences were found between green tea and the placebo for glycosylated hemoglobin (HbA1c), HOMA-IR, fasting insulin, and fasting glucose. In this study, intra-abdominal adipose tissue weight, carcass fat mass, and total fat mass were positively correlated with HOMA-IR and negatively correlated with QUICKI (Fig. 5), suggesting that

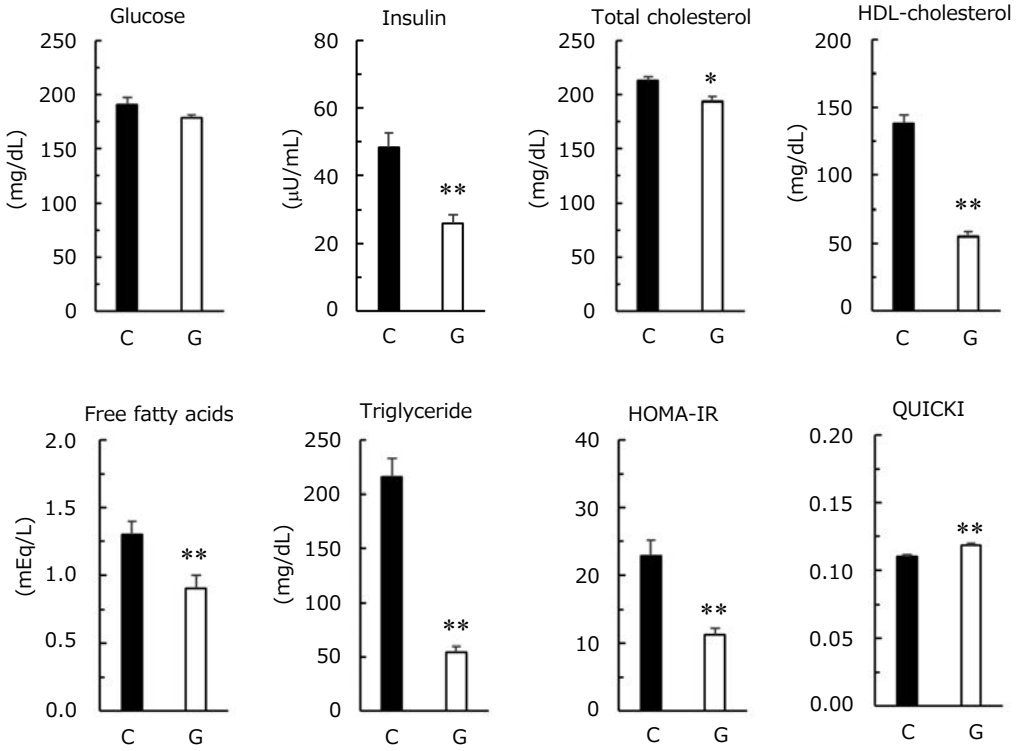

Fig. 3 Concentrations of serum components and insulin resistance indices. HOMA-IR, homeostatic model assessment of insulin resistance; QUICKI, quantitative insulin sensitivity check index. Values are means ± SE for 8 rats. $^{**}p < 0.01$, $^{*}p < 0.05$ vs. group C (Student's t-test).

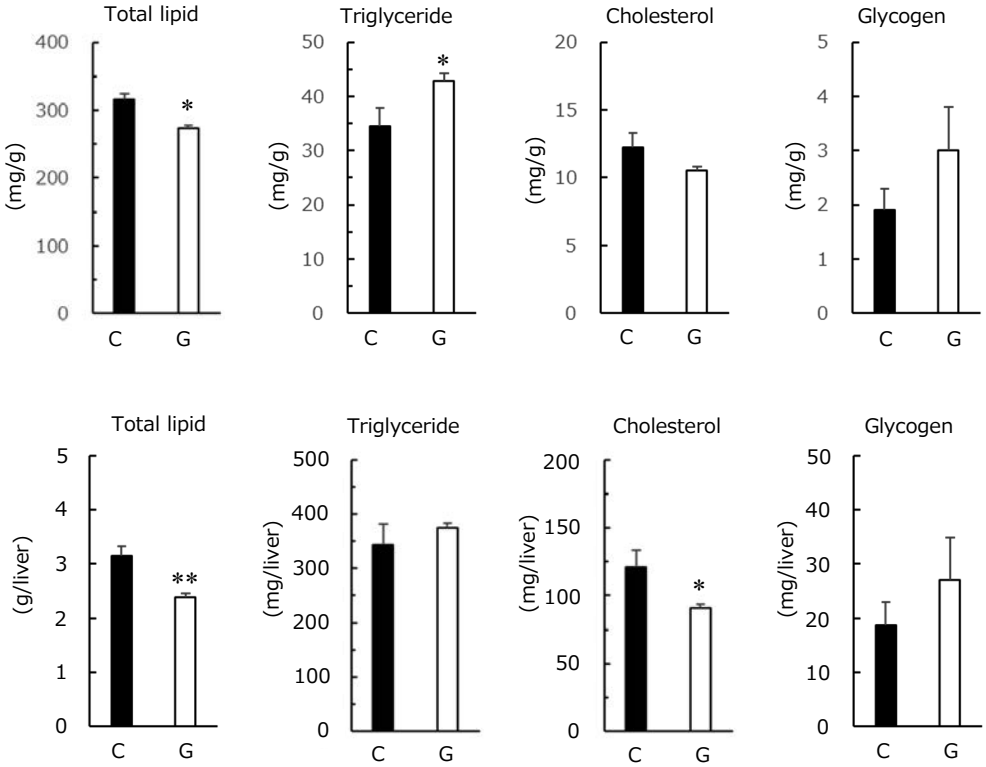

Fig. 4 Contents of liver lipids and glycogen. Values are means ± SE for 8 rats. $^{**}p < 0.01$, $^{*}p < 0.05$ vs. group C (Student's t-test).

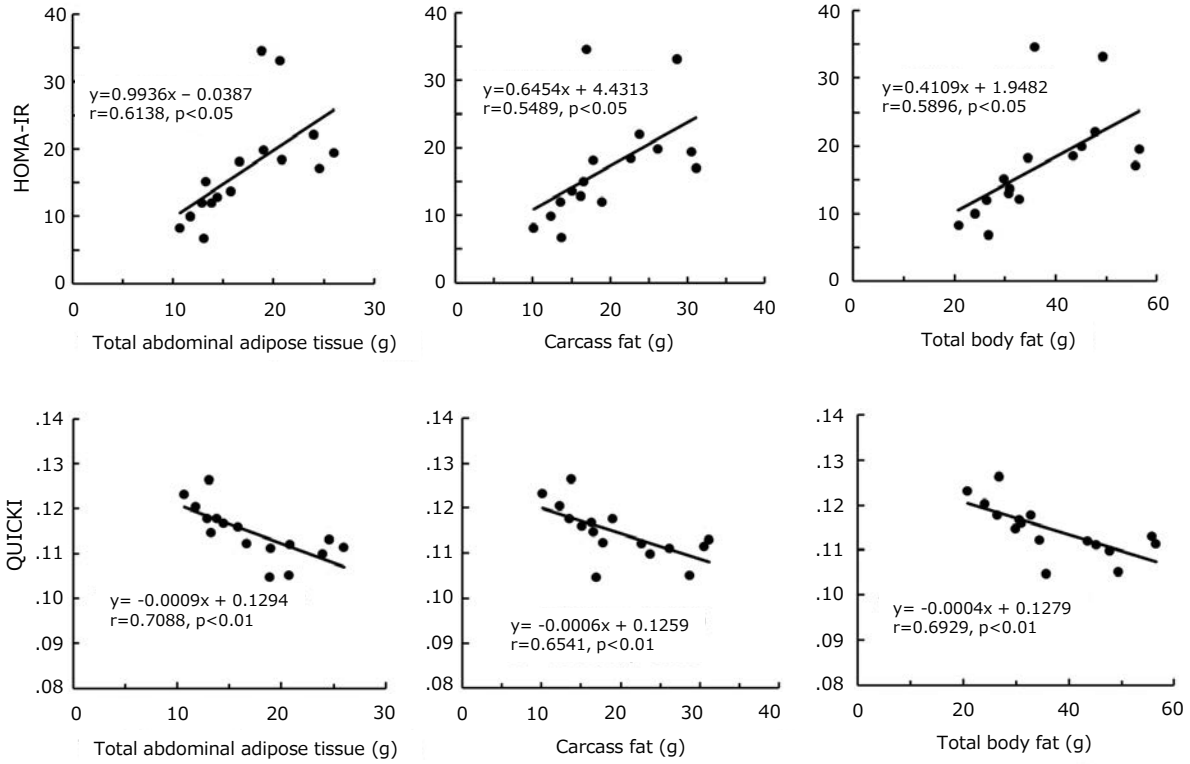

Fig. 5 Correlation of intra-abdominal adipose tissue weight, carcass fat mass, and total fat mass to HOMA-IR and QUICKI.

the beneficial effects of green tea powder on insulin resistance may be a secondary effect of the reduction of body fat accumulation.

In conclusion, we demonstrated that dietary green tea powder supplementation significantly reduced body fat accumulation and improved insulin resistance in rats. Green tea powder is an excellent functional food with anti-obesity effects and can be used as a food.

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緑茶粉末添加食はラットの体脂肪蓄積を低減しインスリン抵抗性を改善する

檜垣俊介[1]・稲井玲子[2]・松尾達博

要　約

肥満は全世界的に重要な健康問題である．緑茶の摂取は肥満の予防・改善に対して非常に有効であることが報告されている．しかしながら，ほとんどの研究は緑茶抽出液あるいは含有抽出成分を実験素材として用いており，緑茶粉末を実験素材として用いた研究は少ない．そこで，本研究では，緑茶粉末添加食をラットに与え，緑茶粉末が体脂肪蓄積とインスリン抵抗性に及ぼす影響について検討した．3週齢Wistar系雄ラットに高脂肪・高ショ糖食（C）あるいは5%緑茶粉末添加食（G）を与え，8週間飼育した．最終体重，体重増加量，食餌摂取量，および食餌効率には2群間に差を認めなかったが，腹腔内脂肪組織重量，屠体脂肪量・脂肪率，および総体脂肪量・脂肪率は，C群に比べてG群で有意に低値を示した．HOMA-IRはC群に比べてG群で有意に低く，QUICKIはC群に比べてG群で有意に高かった．これらの結果から，緑茶粉末添加食はラットの体脂肪蓄積を低減し，インスリン抵抗性を改善することが示唆された．緑茶粉末は抗肥満作用を有する優れた機能性食材であると考えられる．

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