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Modulation of Endocrine Systems and Food Intake by Green Tea Epigallocatechin Gallate*

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ABSTRACT

Green tea polyphenols, especially the catechin, (−)-epigallocatechin gallate (EGCG), have been proposed as a cancer chemopreventative based on a variety of laboratory studies. For clear assessment of the possible physiological effects of green tea consumption, we injected pure green tea catechins ip into rats and studied their acute effects on endocrine systems. We found that EGCG, but not related catechins, significantly reduced food intake; body weight; blood levels of testosterone, estradiol, leptin, insulin, insulin-like growth factor I, LH, glucose, cholesterol, and triglyceride; as well as growth of the prostate, uterus, and ovary. Similar effects were observed in lean and obese male Zucker rats, suggesting that the effect of EGCG was independent of an intact leptin receptor. EGCG may interact specifically with a component of a leptin-independent appetite control pathway. Endocrine changes induced by parenteral administration of EGCG may relate to the observed growth inhibition and regression of human prostate and breast tumors in athymic mice treated with EGCG as well as play a role in the mechanism by which EGCG inhibits cancer initiation and promotion in various animal models of cancer. (*Endocrinology* **141:** 980–987, 2000)

GREEN TEA USE has been linked to a lower incidence of certain cancers and diseases in humans (1). In animal models, long term consumption of green tea polyphenols lowers the incidence of cancers (2) and collagen-induced arthritis (3). *In vitro*, green tea catechins inhibit a variety of enzymes (4–6), are potent antioxidants (7, 8), and alter certain properties of cancer cells in culture (9–11). Whether any of these *in vitro* effects of green tea catechins is responsible for their *in vivo* effects is not clear (12, 13).

We reported previously that ip injection of (−)-epigallocatechin gallate (EGCG), one of the major green tea catechins (Fig. 1), can within 7 days rapidly suppress human prostate and breast tumor growth in athymic mice (14). To assess possible physiological effects of green tea consumption, we studied the acute effects of EGCG on endocrine systems.

Materials and Methods

Animals

Adult Sprague Dawley (Harlan Sprague Dawley, Inc., Indianapolis, IN) rats (male BW, 170–190 g; female BW, 125–145 g) and lean and obese Zucker (15) (Charles River Laboratories, Inc., Wilmington, MA) rats (lean male BW, 240–260 g; obese male BW, 420–440 g) were given free access to a standard rat chow diet and water unless indicated. Animal experimental protocols were approved by the University of Chicago institutional animal care and use committee. Rats were maintained at an ambient temperature of 25 C under a photoperiod of 12 h of light and 12 h of darkness.

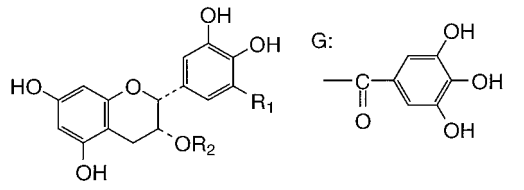
In vivo treatment

EGCG and other catechins (>98% pure) were isolated from green tea (*Camellia sinensis*) in our laboratory as described previously (6). Catechins were dissolved in water for oral administration and in sterile PBS for ip injection. Rats in control groups received vehicle only. Testosterone propionate (TP) and 5α-dihydrotestosterone propionate (DHTP) were dissolved in sesame oil, and 4 mg in 0.5 ml sesame oil (16 mg/kg BW) were injected sc daily when indicated.

Food-restricted, male Sprague Dawley rats were given 12 g rat chow daily, which was about 50% of the amount consumed daily by each control rat. The body weight and the amount of food and water consumed were monitored daily. Food consumption was monitored in rats caged in groups of three to five animals by weighing food pellets every 24 h. On the final day, rats were anesthetized with methoxyflurane, and blood was collected by heart puncture. Sera were collected after centrifugation (10,000 × *g* for 20 min at 4 C) for biochemical analysis.

Biochemical analysis

For biochemical analysis, commercially available RIA kits for insulin-like growth factor I (IGF-I) and testosterone (Diagnostics Systems Laboratories, Inc., Webster, TX), LH and GH (Amersham Pharmacia Biotech, Arlington Heights, IL), leptin and insulin (Linco Research, Inc., St.



	R_1	R_2
(-)Epicatechin (EC)	H	H
(-)Epigallocatechin (EGC)	OH	H
(-)Epicatechin-3-gallate (ECG)	H	G
(-)Epigallocatechin-3-gallate (EGCG)	OH	G

FIG. 1. Structures of four major green tea catechins. The differences among these catechins occur in the number of hydroxyl groups and the presence of a galloyl group.

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Charles, MO), and corticosterone (ICN Biomedicals, Inc., Costa Mesa, CA) and analytical kits for glycerol and triglyceride (Sigma, St. Louis, MO) and fatty acids (Roche Molecular Biochemicals, Indianapolis, IN) were used. Proximate composition analysis of rats was performed by COVANCE Laboratory (Madison, WI). Complete blood count and serum chemistry (*e.g.* cholesterols, glucose, and enzymatic activities) were determined by the Animal Resource Center at the University of Chicago.

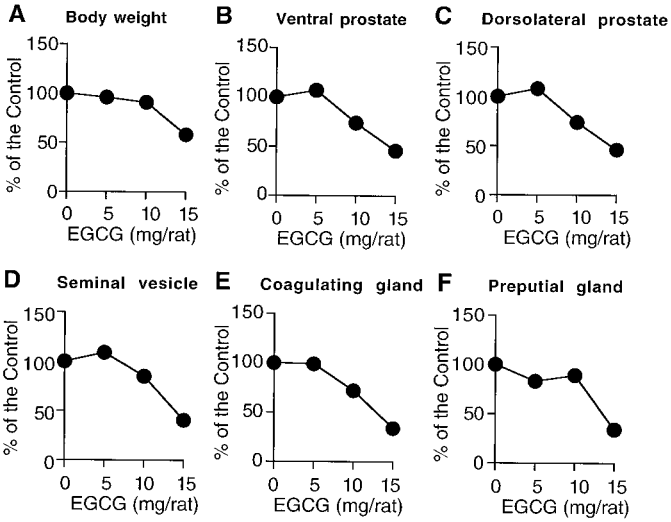

FIG. 2. Dose-dependent effects of EGCG on body weight (A) and weights of the ventral prostate (B), dorsolateral prostate (C), seminal vesicle (D), coagulating gland (E), and preputial gland (F) of male Sprague Dawley rats that were injected ip with the indicated doses of EGCG daily for 7 days. The 5-, 10-, and 15-mg doses of EGCG injected per rat correspond to about 26, 53, and 85 mg/kg BW, respectively. Data are a percentage of the control value calculated from mean values from five animals by comparing body and organ weights of treated rats to those of control rats after 7 days of treatment. The average ending body and organ weights of control rats were: body weight, 243 ± 4 g; ventral prostate, 133 ± 10 mg; dorsolateral prostate, 104 ± 6 mg; seminal vesicle, 171 ± 14 mg; coagulating gland, 51 ± 4 mg; and preputial gland, 119 ± 11 mg. If comparisons are made to starting weights instead of to weights on day 7, the decrease seen with 15 mg EGCG will be smaller. The average starting body and organ weights of control rats were: body weight, 185 ± 4 g; ventral prostate, 123 ± 6 mg; dorsolateral prostate, 91 ± 8 mg; seminal vesicle, 120 ± 12 mg; coagulating gland, 44 ± 2 mg; and preputial gland, 100 ± 15 mg.

Statistical analysis

Data are expressed as the mean ± SEM. Unpaired Student's *t* test was used to examine differences between control and the EGCG-injected groups. ANOVA and Student-Newman-Keuls multiple range test were used to examine differences among various groups. $P < 0.05$ indicated significance.

Results

Body weight

Intraperitoneal injection of EGCG, but not other structurally related green tea catechins, such as EC, EGC, and ECG (Fig. 1), caused acute body weight loss in Sprague Dawley male (Figs. 2A and 3A) and female (Fig. 4A) rats within 2–7 days of treatment. In male Sprague Dawley rats, the effect of EGCG on body weight was dose dependent (Fig. 2). Doses of 5 or 10 mg EGCG (26 and 53 mg/kg BW) injected daily were not effective or were less effective in reducing the body weight than 15 mg (~85 mg/kg BW). Male Sprague Dawley rats injected daily ip with 26 and 53 mg EGCG/kg BW gained body weight by 17–24% relative to their initial body weight, but lost 5–9% relative to the control animals after 7 days of treatment (Fig. 2A). Male Sprague Dawley rats daily injected ip with 85 mg EGCG/kg BW lost 15–21% of their body weight relative to their initial weight and 30–41% relative to the control weight after 7 days of treatment (Figs. 2A and 3A and Table 1). Control rats continued growth and increased their body weight by 25–34% relative to their initial weight (Figs. 2A, 3A, and 4A and Table 1). Female Sprague Dawley rats injected daily ip with 12.5 mg EGCG (~92 mg/kg BW) lost 10% of their body weight relative to their initial weight and 29% relative to the control weight after 7 days of treatment (Fig. 4A). Therefore, an EGCG dose of 70–92 mg/kg BW was used in most experiments.

TABLE 1. EGCG effect on body weight, serum hormones, and organ weight in male Sprague Dawley and Zucker rats

Length of treatment:	Sprague Dawley		Lean Zucker		Obese Zucker	
	7 days		8 days		4 days	
Measurement	Control (10 rats)	EGCG (10 rats)	Control (4 rats)	EGCG (4 rats)	Control (5 rats)	EGCG (5 rats)
Initial BW (g)	182 ± 2	182 ± 1	252 ± 4	248 ± 3	431 ± 14	434 ± 18
Final BW (g)	236 ± 3	154 ± 4	286 ± 4	216 ± 6[a]	463 ± 13	417 ± 18[a]
IGF-I (ng/ml)	2073 ± 174	319 ± 70[a]	1696 ± 54	501 ± 58[a]	1870 ± 89	682 ± 137[a]
Insulin (ng/ml)	1.52 ± 0.25	0.66 ± 0.16[a]	0.91 ± 0.26	0.66 ± 0.05	12.4 ± 2.2	5.46 ± 1.00[a]
Leptin (ng/ml)	1.58 ± 0.16	0.51 ± 0.09[a]	4.84 ± 0.53	1.63 ± 0.64[a]	90.4 ± 0.9	69.2 ± 6.4[a]
Testosterone (ng/ml)	1.77 ± 0.31	0.55 ± 0.36[a]	3.64 ± 0.33	1.03 ± 0.31[a]	2.64 ± 0.26	0.81 ± 0.36[a]
GH (ng/ml)	14.1 ± 3.7	15.5 ± 3.4	9.6 ± 2.1	23.8 ± 14.9	5.1 ± 1.5	17.4 ± 10.0
Corticosterone (ng/ml)	289 ± 33	302 ± 49	351 ± 37	609 ± 101	508 ± 61	360 ± 9
Ventral prostate (mg)	125 ± 12	76 ± 9[a]	280 ± 8	219 ± 10[a]	124 ± 31	96 ± 23
Testis (g)	2.82 ± 0.08	2.70 ± 0.14	ND	ND	ND	ND
Kidney (g)	1.81 ± 0.09	1.44 ± 0.01[a]	1.92 ± 0.02	1.76 ± 0.05[a]	2.51 ± 0.15	2.29 ± 0.01
Liver (g)	10.35 ± 0.19	8.52 ± 0.67[a]	10.68 ± 0.21	8.73 ± 0.24[a]	17.47 ± 1.54	16.71 ± 1.50
Spleen (mg)	693 ± 36	517 ± 60[a]	524 ± 43	449 ± 51	431 ± 17	496 ± 46

The daily dose of EGCG per rat injected was 15 mg (82 mg/kg BW) for Sprague Dawley rats, 20 mg (81 mg/kg BW) for lean Zucker rats, and 40 mg (92 mg/kg BW) for obese Zucker rats. Rats in control groups were injected with PBS without EGCG. Values are the mean ± SEM. ND, Not determined.

[a] Statistically significant ($P < 0.05$) difference between the control and EGCG-injected groups.

Accessory sexual organs and other organs

An effect of EGCG dosage (Fig. 2, B–F) on the weight of accessory sexual organs was also observed. The weights of androgen-sensitive organs, such as ventral (Fig. 2C) and dorsolateral (Fig. 2D) prostates, seminal vesicles (Fig. 2E), coagulating glands (Fig. 2F), and preputial glands (Fig. 2F) were reduced by 50–70% after 7 days of treatment with EGCG (~85 mg/kg BW). Weight changes in these sexual organs were modulated in a catechin-specific manner (Fig. 3, C–F). Relative to control animals killed at the start of the experiment, these accessory sexual organs (except preputial gland) in male Sprague Dawley rats were reduced by 30–50% in weight after 7 days of EGCG treatment (Fig. 2, B–F). Similarly, the weights of estrogen-sensitive organs, such as the uterus (Fig. 4C) and ovary (Fig. 4D), in female Sprague Dawley rats were reduced by about 50% after 7 days of EGCG treatment. The weights of liver and kidney were also decreased by about 20% (data not shown). In male Sprague Dawley and lean Zucker rats treated with EGCG for 7–8 days, the weights of the liver, kidney, and testis were reduced by about 10–20%, whereas spleen weight was reduced by about 15–30% (Table 1). However, there was no change in

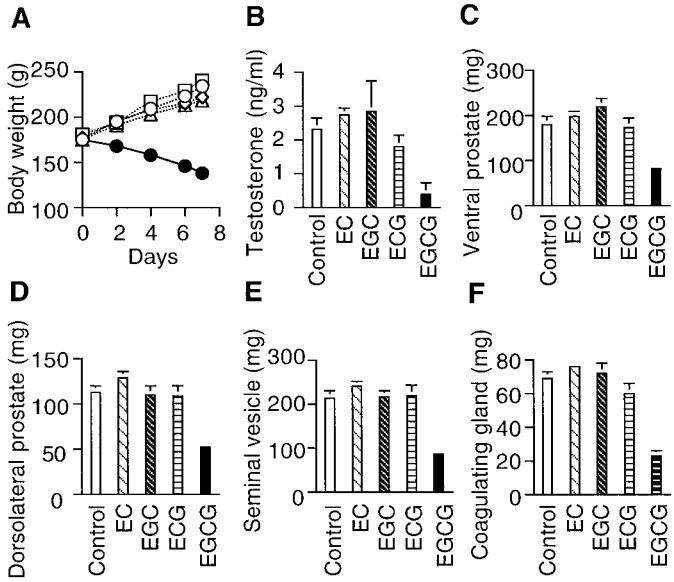

FIG. 3. Differential effects of EGCG and three related green tea catechins on body weight (A), serum testosterone (B), and weights of the ventral prostate (C), dorsolateral prostate (D), seminal vesicle (E), and coagulating gland (F) in male Sprague Dawley rats. Rats were injected ip with the indicated catechin, 15 mg/rat (85 mg/kg BW), daily for 7 days. Values are the mean ± SEM from five animals in each group. The SE bar is either too small to be seen or, for clarity, is not shown. Symbols in A correspond to control (○), EC (◇), EGC (□), ECG (△), and EGCG (●) groups.

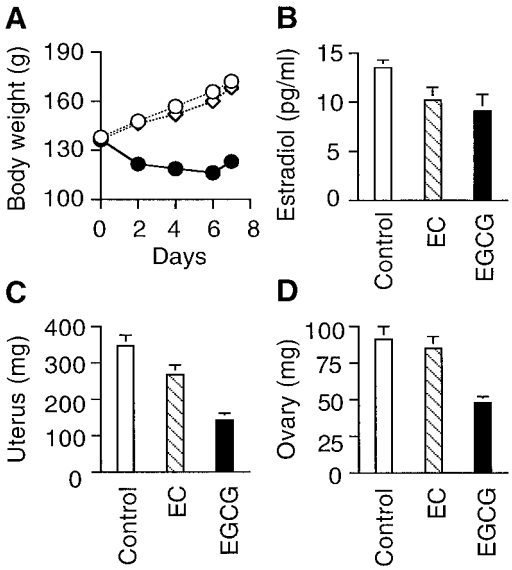

FIG. 4. Differential effects of EGCG and three related green tea catechins on body weight (A), serum 17β-estradiol (B), and weights of the uterus (H), and ovary (I) in female Sprague Dawley rats. Rats were injected ip with the indicated catechin, 12.5 mg/rat (92 mg/kg BW), daily for 7 days. Values are the mean ± SEM from five animals in each group. The SE bar is either too small to be seen or, for clarity, is not shown. Symbols in A correspond to control (○), EC (◇), and EGCG (●) groups.

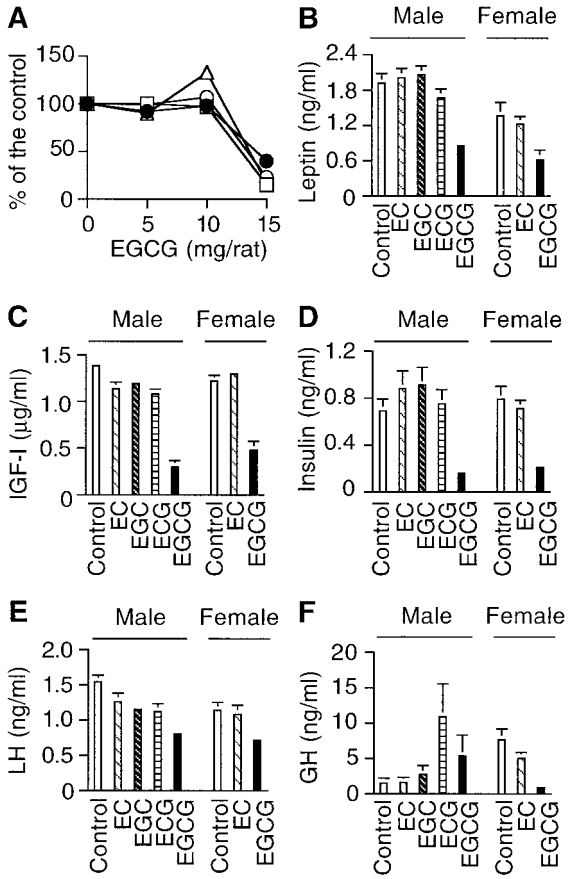

FIG. 5. The effects of EGCG dosage and different catechins on hormone levels of Sprague Dawley rats. Male rats were injected ip with the indicated doses of EGCG (5 mg/rat, 26 mg/kg BW; 10 mg/rat, 53 mg/kg BW; 15 mg/rat, 85 mg/kg BW) daily for 7 days, and serum levels of leptin (●), IGF-I (□), insulin (○), and testosterone (△) were measured (A). Male and female rats were injected ip with the indicated catechin (15 mg for male, 85 mg/kg BW; 12.5 mg for female, 92 mg/kg BW) daily for 7 days, and serum levels of leptin (B), IGF-I (C), insulin (D), LH (E), and GH (F) were measured. Values are the mean ± SEM from five animals in each group.

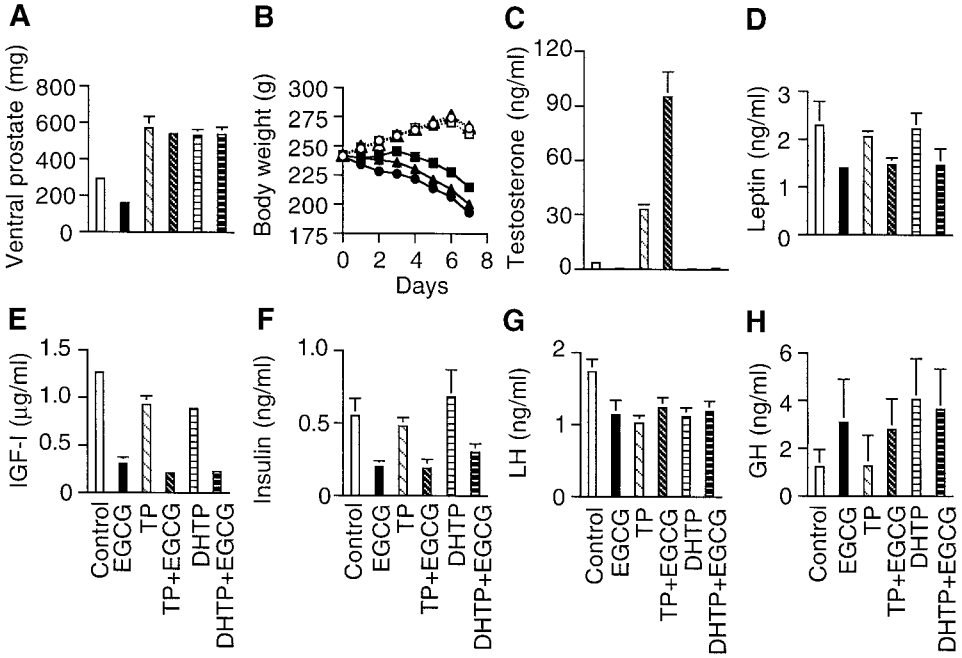

FIG. 6. Effect of exogenous androgen on EGCG-induced reduction of body and prostate weight and serum hormones in male Sprague Dawley rats. Rats (initial weight, 235–245 g) were injected ip with 20 mg EGCG (81–85 mg/kg BW) and/or 4 mg TP or DHTP (16 mg/kg BW) daily for 7 days. Then ventral prostate (A) and body (B) weights were determined, and blood was collected for analysis of serum testosterone (C), leptin (D), IGF-I (E), insulin (F), LH (G), and GH (H). The SE bar is either too small to be seen or, for clarity, is not shown. Symbols in B correspond to control (○), EGCG (●), TP (□), TP plus EGCG (■), DHTP (△), and DHTP plus EGCG (▲) groups.

these organ weights from those in male obese Zucker rats treated with EGCG for 4 days (Table 1).

Sex hormones, leptin, IGF-I, insulin, LH, and GH

Rats treated with EGCG had significant changes in various endocrine parameters. After 7 days of treatment with EGCG (~85 mg/kg BW) circulating testosterone (Fig. 3B and Table 1) was reduced by about 70% in male Sprague Dawley rats. Similarly, the circulating level of 17β-estradiol was reduced by 34% (Fig. 4B) in females after 7 days of EGCG treatment. In both male and female Sprague Dawley rats, 7 days of EGCG treatment caused significant reduction in blood levels of leptin, IGF-I, and insulin (Fig. 5, A–D, and Table 1). Dose-dependent effects of EGCG in male Sprague Dawley rats were also observed on levels of serum testosterone, leptin, IGF-I, and insulin (Fig. 5A). With male and female Sprague Dawley rats treated with EGCG for 7 days, we also observed that the serum level of LH was significantly reduced (40–50%; Fig. 5E), whereas that of GH was increased in males or reduced in females (Fig. 5F). However, the pulsatile nature of GH secretion prevented us from making definite conclusions about changes in circulating levels of GH in these rats. The effects of EGCG on sex hormones and various peptide hormones investigated was not mimicked by other structurally similar catechins (*i.e.* ECG with one less hydroxyl group than EGCG was not active; Fig. 5).

Lean and obese male Zucker rats treated with EGCG also showed similar changes in the serum levels of testosterone, leptin, IGF-I, insulin, and GH and prostate weight (Table 1). For both Sprague Dawley and Zucker rats, significant effects were observed with 70–92 mg EGCG/kg BW.

Exogenous androgen reverses the effect of EGCG on accessory sexual organs

To determine whether the reduction in weight of accessory sexual organs was due to an EGCG-induced reduction in androgen levels, we injected male Sprague Dawley rats with androgen and/or EGCG. We found that EGCG did not cause prostate weight loss in male rats injected daily with TP or DHTP (Fig. 6A); therefore, the EGCG effect on prostate weight was most likely secondary to the EGCG-induced reduction in the level of testosterone in these male rats. However, androgen administration was not able to prevent the EGCG-induced body weight loss (Fig. 6B); food intake restriction (Fig. 7E); decreases in circulating leptin (Fig. 6D), IGF-I (Fig. 6E), insulin (Fig. 6F), and LH (Fig. 6G); or increase in circulating GH (Fig. 6H).

Serum nutrients and proximate body composition

In EGCG-treated male Sprague Dawley rats, the serum levels of protein, fatty acids, and glycerol were not altered, but significant reductions in serum glucose (−32%), lipids (−15%), triglycerides (−46%), and cholesterol (−20%) were observed (Table 2). Similar changes in these serum nutrients were observed in male lean and obese Zucker rats. Proximate composition analysis of animals showed that Sprague Dawley rats treated daily with EGCG for 7 days had no change in percent water and protein content, a moderate decrease in carbohydrate content (2.5% in control and 1.3% in the EGCG-treated group), but a very large reduction in fat content (from 4.1% in controls to 1.4% in the EGCG-treated group). Within 7–8 days, EGCG treatment decreased sc fat by 40–70% and abdominal fat by 20–35%, but not epididymal fat, in male Sprague Dawley and lean Zucker rats (Table 2). A 20% loss of abdominal fat was seen in obese male Zucker rats within 4 days of EGCG treatment (Table 2).

Effect of EGCG on food intake

We found that EGCG-treated Sprague Dawley male (Fig. 7, A and B) and female (Fig. 7C) rats consumed about 50–60% less food than control rats. Similar effects of EGCG on food

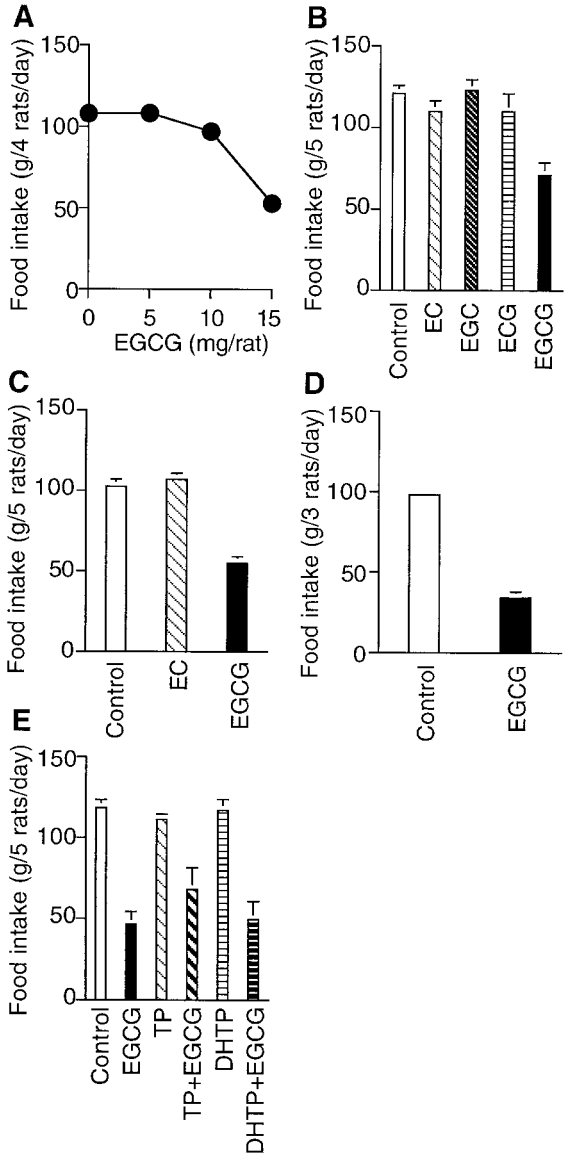

FIG. 7. Effect of green tea catechins on food intake in male Sprague Dawley and obese Zucker rats. A, Male Sprague Dawley rats were injected ip with the indicated doses of EGCG (5 mg/rat, 26 mg/kg BW; 10 mg/rat, 53 mg/kg BW; 15 mg/rat, 85 mg/kg BW) daily for 7 days. B, Male Sprague Dawley rats were injected ip with 15 mg of the indicated green tea catechins (85 mg/kg BW) daily for 7 days. C, Female Sprague Dawley rats were injected ip with 12.5 mg of either EC or EGCG (92 mg/kg BW) daily for 7 days. D, Male obese Zucker rats were injected ip with 30 mg EGCG/rat (92 mg/kg BW) daily for 8 days. E, Effect of exogenous androgen on EGCG-induced reduction in food intake. Male Sprague Dawley rats were injected daily for 7 days with 20 mg EGCG (83 mg/kg BW, ip) and/or 4 mg of the indicated androgen (16 mg/kg BW, sc). Values are the mean ± SEM from five animals in each group.

intake were observed with obese male Zucker rats (Fig. 7D). Therefore, body weight loss was due to reduced intake of food. As food restriction can alter hypothalamic function and decrease the level of LH and sex steroids (16, 17), we restricted the food intake of Sprague Dawley male rats (not injected with EGCG) by about 50% for 7 days and found that the blood level of testosterone was indeed reduced by about 60% and ventral prostate weight was decreased by about 50% compared with those in animals given free access to food (Table 3). Serum leptin, IGF-I, insulin, LH, and GH were also decreased after food restriction. Administration of androgen to male Sprague Dawley rats was not able to prevent the EGCG-induced food intake reduction (Fig. 7E). These effects of EGCG, administered ip, were diminished or absent when EGCG was administered orally (Table 3).

Blood chemistry and blood cell composition

Male Sprague Dawley rats were treated with EGCG and ECG for 7 days and then their serum and whole blood were analyzed for various components (Tables 4 and 5). Neither EGCG nor structurally related ECG caused significant changes in the serum levels of total protein, albumin, blood urea nitrogen, creatine, PO_4^{3-}, Na^+, K^+, Ca^{2+}, Cl^-, and enzymes that are indicative of severe damage to liver and other organs, such as lactate dehydrogenase, alanine aminotransferase, aspartate aminotransferase, and γ-glutamyltranspeptidase (Table 4). However, significant changes in the amount of blood bilirubin and the activity of blood alkaline phosphatase were observed. In blood of rats treated with EGCG, red blood cell and hemoglobin concentrations increased by about 20%, whereas the concentrations of white blood cells, lymphocytes, and monocytes decreased about 10%, 31%, and 24%, respectively (Table 5). Both eosinophil and platelet concentrations increased by 100%.

Discussion

The present report describes catechin-specific modulation of endocrine systems in rats. The effects of the green tea catechin, EGCG, were dose dependent and gender and strain independent. In addition, differential effects of green tea catechins on body weight loss, food intake restriction, decreases in accessory sexual organ weight, and decreases in blood nutrients were observed. The effect of EGCG on the weight of male accessory sexual organs was due to lowered circulating levels of testosterone. This conclusion is supported by the following observations. 1) Androgens such as TP and DHTP blocked the effect of EGCG on the weight of accessory sexual organs, including prostates. 2) EGCG did not reduce prostate weight in androgen-supplemented castrated Sprague Dawley rats (our unpublished observations). 3) EGCG-induced weight loss of prostate and other androgen-sensitive organs was accompanied by an EGCG-induced lowering of serum testosterone. Previous reports have suggested that EGCG *in vitro* controls prostate cell growth by inducing apoptosis (10); however, our *in vivo* studies suggest that complex endocrine changes may be responsible for EGCG-induced human prostate cancer regression in nude mice (14).

The effects of EGCG on body weight loss, hormone level changes, and food intake depend on the route of administration. The effects of EGCG were not observed or were less when the same amount of EGCG was given to rats orally for 7 days. This may be due to inefficient absorption of EGCG (13, 18, 19) and suggests that the effects of EGCG administered ip were not caused by interaction of EGCG with food or by EGCG action inside the gastrointestinal tract.

TABLE 2. EGCG effect on serum nutrients and fat tissues in male Sprague Dawley and Zucker rats

Length of treatment:	Sprague Dawley		Lean Zucker		Obese Zucker	
	7 days		8 days		4 days	
Measurement	Control (10 rats)	EGCG (10 rats)	Control (4 rats)	EGCG (4 rats)	Control (5 rats)	EGCG (5 rats)
Glucose (mg/dl)	176 ± 14	120 ± 12[a]	169 ± 17	122 ± 11[a]	190 ± 16	122 ± 16[a]
Protein (g/dl)	5.6 ± 0.1	5.5 ± 0.1	5.5 ± 0.1	5.3 ± 0.3	6.0 ± 0.1	5.3 ± 0.3
Lipid (mg/dl)	527 ± 22	447 ± 16[a]	1213 ± 83	877 ± 77[a]	2632 ± 296	1501 ± 289[a]
Triglyceride (mg/dl)	48 ± 5	26 ± 3[a]	87 ± 4	49 ± 7[a]	262 ± 25	171 ± 26[a]
Cholesterol (mg/dl)	83 ± 0.8	69 ± 2.2[a]	78 ± 4	61 ± 5[a]	152 ± 11	121 ± 4[a]
Fatty acid (μmol/ml)	1.58 ± 0.08	1.63 ± 0.27	ND	ND	ND	ND
Glycerol (mg/dl)	0.99 ± 0.17	0.82 ± 0.14	20.2 ± 2.1	14.5 ± 1.3	99.3 ± 9.9	45.6 ± 7.8[a]
Subcutaneous fat (g)	9.77 ± 0.59	2.08 ± 0.23[a]	18.88 ± 0.66	7.85 ± 1.23[a]	115 ± 3	110 ± 3
Abdominal fat tissue (g)	ND	ND	2.33 ± 0.22	1.52 ± 0.19[a]	15.32 ± 0.80	12.31 ± 0.67[a]
Epididymal fat tissue (g)	1.32 ± 0.07	1.47 ± 0.16	2.50 ± 0.14	2.82 ± 0.23	9.92 ± 0.80	9.93 ± 0.98

Body weights are the same as in Table 1. The daily dose of EGCG per rat for injection was 15 mg (82 mg/kg BW) for Sprague-Dawley rats, 20 mg (81 mg/kg BW) for lean Zucker rats, and 40 mg (92 mg/kg BW) for obese Zucker rats. Rats in control groups were injected with PBS without EGCG. Values are the mean ± SEM. The amount of sc fat was estimated from proximate analysis of rat carcasses and assuming all fat was derived from sc sources. Abdominal fat represents dissected peritoneal fat excluding epididymal fat. ND, Not determined.

[a] Statistically significant ($P < 0.05$) difference between the control and EGCG-injected groups.

TABLE 3. A comparison of orally and ip administered EGCG and 50% food restriction on serum hormones, body weight, food intake, and organ weight in male Sprague Dawley rats

	Oral		50% Food	ip	
	Control	EGCG		Control	EGCG
BW (g)					
Initial	188.4 ± 2.98	184.2 ± 2.3	184.2 ± 3.7	174.8 ± 2.2	174.8 ± 3.8
Final	238.8 ± 4.0	228.0 ± 3.8	168.2 ± 1.4[a]	234.5 ± 3.1	138.0 ± 6.3[a]
Food intake (g/5 rats·day)	135.5 ± 4.9	115.8 ± 6.4[a]	60 ± 0	121.2 ± 5.1	63.7 ± 4.1[a]
Testosterone (ng/ml)	2.88 ± 0.48	2.59 ± 0.74	1.19 ± 0.35[a]	2.32 ± 0.33	0.43 ± 0.33[a]
IGF-I (ng/ml)	1341 ± 59	1189 ± 14	556 ± 42[a]	1384 ± 45	300 ± 68[a]
Insulin (ng/ml)	0.36 ± 0.05	0.34 ± 0.08	0.12 ± 0.05[a]	0.67 ± 0.11	0.16 ± 0.01[a]
Leptin (ng/ml)	1.85 ± 0.14	1.21 ± 0.06[a]	0.70 ± 0.04[a]	1.91 ± 0.17	0.85 ± 0.07[a]
LH (ng/ml)	1.57 ± 0.11	1.17 ± 0.04[a]	1.19 ± 0.09[a]	1.54 ± 0.01	0.72 ± 0.04[a]
GH (ng/ml)	2.81 ± 1.63	3.36 ± 1.13	1.17 ± 0.42[a]	1.50 ± 0.73	5.32 ± 2.95
Ventral prostate (mg)	206.4 ± 15.5	201.1 ± 8.8	112.4 ± 7.7[a]	180.4 ± 17.0	82.2 ± 8.3[a]
Dorsolateral prostate (mg)	126.5 ± 5.7	123.8 ± 7.4	82.9 ± 9.4[a]	113.0 ± 6.7	52.5 ± 5.3[a]
Seminal vesicle (mg)	207.8 ± 13.4	198.5 ± 12.7	116.4 ± 15.6[a]	213.7 ± 15.3	86.8 ± 9.2[a]
Coagulating gland (mg)	80.2 ± 6.3	65.4 ± 4.7	35.7 ± 2.5[a]	69.4 ± 4.2	23.5 ± 3.2[a]
Preputial gland (mg)	108.3 ± 6.1	126.8 ± 6.2	69.0 ± 6.7[a]	104.9 ± 17.3	35.8 ± 5.8[a]
Testis (g)	3.10 ± 0.11	3.11 ± 0.05	2.86 ± 0.11[a]	3.04 ± 0.09	2.54 ± 0.09[a]
Kidney (g)	1.85 ± 0.04	1.75 ± 0.07	1.27 ± 0.01[a]	1.84 ± 0.01	1.36 ± 0.07[a]

Values are the mean ± SEM for four or five determinations. Control groups were treated with vehicle. Male Sprague Dawley rats were given 15 mg EGCG/rat (orally, 81 mg/kg BW; ip, 85 mg/kg BW) daily for 7 days either orally or injected intraperitoneally. Another group of rats was subjected to approximately 50% food restriction.

[a] Statistically significant ($P < 0.05$) difference between the control and EGCG-injected groups or between the oral control and 50% food restriction groups.

We have determined the plasma concentration of EGCG by HPLC (20) and have also found that after ip injection of Sprague Dawley rats with 100 mg EGCG/kg BW, plasma EGCG levels were 24, 2, 4, 1, and 1 μM at 0.5, 1, 2, 5, and 24 h, respectively (average of three rats). Therefore, EGCG may have systemic effects in this study. A plasma EGCG concentration of 1 μM would be similar to levels in humans (70 kg) 1 h after drinking 6–12 cups (200 ml/cup) of tea (18).

The biological effects of EGCG have often been attributed to its *in vitro* effects on different enzyme activities (4–6), cell proliferation (9–11), and transcriptional activators (1) as well as its antioxidant and free radical-scavenging activity (7, 8). The effects of EGCG on various endocrine parameters that we have observed may be explained as secondary effects of EGCG on food intake. For example, the large decrease in circulating leptin in EGCG-treated rats could have been caused by diminished fat stores due to low food intake in these rats. Both glucose and insulin stimulate leptin gene expression (21, 22); therefore, low circulating levels of glucose and insulin, possibly resulting from low food intake, may also have contributed to the effect of EGCG on the leptin level. However, other mechanisms for the effects of EGCG, besides lowering food intake, should be explored.

The effect of EGCG, but not those of other related catechins, on food intake is interesting. A 50% decrease in food intake was seen by the second day of treatment with 80 mg EGCG/kg BW. The EGCG effect on food intake was not dependent on an intact leptin receptor, as the leptin receptor-defective obese Zucker rats also responded to EGCG. EGCG may interact specifically with a component of a leptin receptor-independent appetite control pathway and reduce food intake. As food intake is regulated by a variety of

TABLE 4. Differential effects of ECG and EGCG on serum chemistry of male Sprague Dawley rats

Serum characteristics	Control	ECG	EGCG
Protein (g/dl)	5.6 ± 0.1	5.8 ± 0.1	5.5 ± 0.1
Albumin (g/dl)	3.25 ± 0.04	3.17 ± 0.04	3.04 ± 0.12
Bilirubin (mg/dl)	0.12 ± 0.05^a	0.19 ± 0.01^a	0.27 ± 0.04^b
BUN (mg/dl)	20 ± 1.41	20.75 ± 0.75	22.43 ± 2.17
Creatine (mg/dl)	0.60 ± 0.04	0.55 ± 0.03	0.5 ± 0
PO_4^{-3} (mg/dl)	11.22 ± 0.92	9.68 ± 0.62	9.22 ± 1.24
Na^+ (mM)	139.25 ± 0.70	138.37 ± 0.82	136.57 ± 0.95
K^+ (mM)	4.59 ± 0.19	5.01 ± 0.13	4.96 ± 0.07
Ca^{+2} (mg/dl)	10.17 ± 0.37	9.71 ± 0.20	9.46 ± 0.52
Cl^- (mM)	93.37 ± 0.73	93.00 ± 0.60	91.43 ± 1.06
LDH (U/liter)	927 ± 83	1069 ± 106	1199 ± 78
ALT (U/liter)	58 ± 2	53 ± 3	52 ± 3
AST (U/liter)	173 ± 17	188 ± 17	200 ± 13.82
GGT (U/liter)	2.50 ± 0.71	3.12 ± 0.72	3.71 ± 0.42
ALKP (U/liter)	171 ± 7^a	136 ± 18^{a,b}	129 ± 9^b

Data are expressed as the mean ± SEM for eight determinations. Rats were injected ip with 15 mg ECG or EGCG (82 mg/kg BW) daily for 7 days. For each serum parameter, *different letters* represent a significant ($P < 0.05$) difference between two groups after one-way ANOVA and Student-Newman-Keuls multiple range test. ALT, Alanine transaminase; AST, aspartate aminotransferase; GGT, γ-glutamyltransferase; ALKP, alkaline phosphatase; LDH, lactate dehydrogenase; BUN, blood urea nitrogen.

TABLE 5. Differential effects of ECG and EGCG on blood cell composition of male Sprague Dawley rats

Blood characteristics	Control	ECG	EGCG
Hematocrit (%)	40.9 ± 1.2^a	45.4 ± 0.9^b	45.9 ± 1.0^b
Erythrocyte parameter			
RBC ($\times 10^6/\mu$l)	6.51 ± 0.11^a	7.40 ± 0.23^{a,b}	7.90 ± 0.15^b
Hb (g/dl)	13.7 ± 0.46^a	15.5 ± 0.45^b	16.8 ± 0.46^b
MCV (fl)	62.8 ± 0.7^a	61.4 ± 0.7^a	58.1 ± 0.4^b
MCH (pg)	21.0 ± 0.3	20.9 ± 0.2	21.2 ± 0.2
MCHC (g/dl)	33.4 ± 0.2^a	34.1 ± 0.3^a	36.5 ± 0.2^b
Reticulocytes (%)	3.2 ± 1.2	5.0 ± 1.2	2.2 ± 1.1
Leukocyte parameter			
WBC ($\times 10^3/\mu$l)	11.03 ± 1.39	9.40 ± 1.00	9.92 ± 0.19
Neutrophil	1177 ± 194	3375 ± 490	2875 ± 788
Lymphocyte	9400 ± 1422^a	5800 ± 511^b	6475 ± 578^b
Monocyte	520 ± 63^a	207 ± 20^b	397 ± 73^a
Eosinophil	115 ± 25	100 ± 11	230 ± 73
Platelet ($\times 10^3/\mu$l)	950 ± 80^a	1325 ± 96^b	1949 ± 59^c

Data are expressed as the mean ± SEM for three or four determinations. Rats were injected ip with 15 mg ECG or EGCG (82 mg/kg BW) daily for 7 days. For each blood parameter, *different letters* represent a significant ($P < 0.05$) difference between two groups after one-way ANOVA and Student-Newman-Keuls multiple range test. RBC, Red blood cell; Hb, hemoglobulin; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobulin; MCHC, mean corpuscular hemoglobulin content; WBC, white blood cell.

peripheral factors and by central neuroendocrine systems (23, 24), we measured plasma levels of peptides, such as ACTH, neuropeptide Y, CRF, urocortin, and galanin, in male Sprague Dawley rats after they were treated with 83 mg EGCG/kg BW for 2 days. EGCG did not change plasma levels of these neuropeptides (our unpublished observations). Whether hypothalamic neuropeptide gene expression is altered by EGCG is being investigated. Various hormones, including cholecystokinin, glucagon-like polypeptide-1, glucagon, substance P, somatostatin, and bombesin, have been reported to inhibit food intake (23, 24). Further study is required to determine whether any of these components is responsible for the effect of EGCG on food intake.

EGCG does not appear to be toxic to the liver and kidney, as 1) EGCG did not cause significant changes in the serum level of total protein, albumin, blood urea nitrogen, creatine, PO_4^{3-}, Na^+, K^+, Ca^{2+}, Cl^-, and enzymes that are indicative of severe damage to liver and other organs; 2) EGCG had no effect on male Sprague Dawley rat liver ornithine decarboxylase activity (an indicator of cell proliferation that increases upon liver damage) (25); and 3) in lean and obese Zucker rats, we did not observe any visible differences between microscopic histology of the liver and kidney of EGCG-treated rats and those of the controls. Although no statistically significant elevation of serum aspartate aminotransferase and γ-glutamyltranspeptidase activity was observed in EGCG-treated rats, the small increase in the activities of these enzymes in serum may be indicative of an effect of EGCG on liver or may be related to lowered food intake (26). Significant changes in serum bilirubin and alkaline phosphatase activity in EGCG-injected rats may also be related to diet restriction (26, 27). Although detailed toxicological studies of EGCG have not been reported, a condensed polyphenol structurally related to EGCG, procyanidin B-2, has a lethal dose greater than 2000 mg/kg BW when sc injected into rats (28).

Although oral administration of EGCG was not effective within 7–14 days, long term oral consumption of green tea or EGCG-containing extracts may mimic some of the acute

EGCG effects described in this report and may be beneficial to health. Studies have shown that oral consumption of green tea or EGCG can lower rat and human serum cholesterol levels (29–31), increase rat high density lipoprotein cholesterol (30), decrease rat and human low density lipoprotein cholesterol (30, 31), and lower rat blood glucose (32) and triglyceride (30). Based on oral and ip effects of EGCG on serum hormones and nutrients, long term consumption of green tea may influence the incidence of obesity, diabetes, and cardiovascular disease. Recently, it was shown that EGCG inhibits angiogenesis (33), which may relate to the effects of EGCG on tumor growth (14). Also, by lowering plasma levels of sex steroids and other endocrine factors, such as IGF-I, long term use of EGCG or green tea may be effective in the prevention and suppression of the growth of hormone-dependent and -independent prostate and breast cancer (14, 34, 35). This may relate to the low occurrence of breast and prostate cancer metastasis and mortality in some Asian countries (14, 36) where green tea is consumed regularly. Despite many potential benefits of green tea and EGCG consumption, it is also important to evaluate undesirable health-related consequences that may arise from EGCG-induced reductions in the levels of sex steroid hormones and other endocrine factors.

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