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Review

Tea, obesity, and diabetes

Yung-Hsi Kao, Hsin-Huei Chang, Meng-Jung Lee and Chia-Lin Chen

Department of Life Science, College of Science, National Central University, Chung-Li City, Taoyuan, Taiwan

Tea has been found to possess widespread biological functions based on a variety of laboratory data. The effects of tea on obesity and diabetes have received increasing attention. This paper reviews the evidence for the connections among tea catechins, and obesity and diabetes. Tea catechins, especially (−)-epigallocatechin gallate (EGCG), appear to have antiobesity and antidiabetic effects. While few epidemiological and clinical studies show the health benefits of EGCG on obesity and diabetes, the mechanisms of its actions are emerging based on the various laboratory data. These mechanisms may be related to certain pathways, such as through the modulations of energy balance, endocrine systems, food intake, lipid and carbohydrate metabolism, the redox status, and activities of different types of cells (*i.e.*, fat, liver, muscle, and β-pancreatic cells). Because the EGCG receptor, the so-called 67-kDa laminin receptor (LR), has been discovered with colocalization of other types of LR and cytoskeleton in both cancer cells and normal cells, this may explain that EGCG possesses numerous actions. The mechanistic results of this review may possibly be utilized in the treatment of obesity, diabetes, and other related diseases using tea- and EGCG-based folk medicines.

Keywords: Diabetes / Epigallocatechin gallate / Kinase / Obesity / Tea

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1 Introduction

Obesity and diabetes are two common diseases associated with each other and with risks of cancer, hypertension, neurological disorders, and cardiovascular diseases [1, 2]. Maybe for this reason, estimates of the economic costs, prevalence, morbidity, and mortality associated with more modest degrees of being overweight, obese, and having diabetes are rising [3–6]. Development of obesity and diabetes in an individual is, respectively, characterized by increased number and size of fat cells and by elevated blood glucose levels. They are regulated by genetic, endocrine, metabolic, neurological, pharmacological, environmental, and nutritional factors [7]. Accordingly, a better understanding of the mechanism through which a particular dietary nutrient modulates obesity and diabetes would be of great benefit to persons who are undergoing initiation and progression of both diseases and associated morbidities.

Tea is the most widely consumed beverage in the world. It is believed to have medicinal efficacy in the prevention and treatment of many diseases, and so longevity is often associated with the habit of drinking tea [8–13]. However, scientific and medical evaluation of tea began only recently according to a literature survey from PubMed (Fig. 1). Since 1995, increasing number of publications can be found on green, oolong, and black tea, catechins, and (−)-epigallocatechin gallate (EGCG), which is one of the major green tea catechins considered as a cancer, obesity, diabetes, and cardiovascular disease chemopreventative agent [8–13]. This reflects increased research on the possible health benefits of tea beverage [8–13].

Tea catechins are polyphenolic flavonoids which were once called vitamin P [14]. Since the discoveries that they have unique chemical structures and are major ingredients of unfermented and semifermented tea [15], they have been

Correspondence: Dr. Yung-Hsi Kao, Department of Life Science, College of Science, National Central University, Chung-Li City, Taoyuan, Taiwan 320
E-mail: ykao@cc.ncu.edu.tw
Fax: +886-3-4228482

Abbreviations: **ACC**, acetyl-CoA carboxylase; **BAT**, brown adipose tissue; **COMT**, catechol-*O*-methyltransferase; **Dex**, dexamethasone; **EC**, (−)-epicatechin; **ECG**, (−)-epicatechin gallate; **EGC**, (−)-epigallocatechin; **EGCG**, (−)-epigallocatechin gallate; **FAS**, fatty acid synthase; **G3PDH**, glycerol-3-phosphate dehydrogenase; **G6PDH**, glucose-6-phosphate dehydrogenase; **GLUT**, glucose transporter; **HDL**, high-density lipoprotein; **LDL**, low-density lipoprotein; **LR**, laminin receptor; **ME**, malic enzyme; **MIX**, 1-methyl-3-isobutylxanthine; **PL**, pancreatic lipase; **Rstn**, resistin; **UCP**, uncoupling protein; **STZ**, streptozotocin

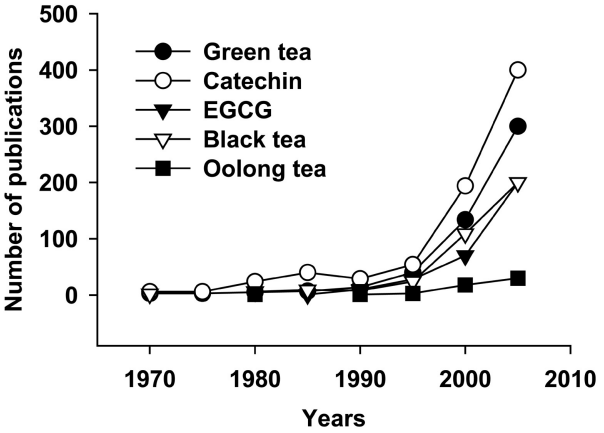


Figure 1. Yearly number of publications related to green, oolong, and black tea, catechins, and EGCG from 1970 to 2005 in 5–year intervals. Data were obtained from Pubmed. Data for the year 2005 are projected from the number available for the first 6 months of 2005.

found to possess widespread biological functions and health benefits [8–13]. For example, tea catechins, especially EGCG, lower the incidence of cancers [8–13], collagen-induced arthritis [16], oxidative stress-induced neurodegenerative diseases [17], and cytokine-induced inflammation *in vivo* [8]. Also, EGCG can reduce body weight and body fat [18, 19]. In addition, the roles of EGCG in the regulation of diabetes and blood glucose levels [20] are beginning to emerge. Despite the importance of EGCG and other tea catechins, relatively little is known about the mechanism of their action in regulating body weight and diabetes when compared to their anticancer effects [8–13]. The fact that the EGCG receptor, the so-called 67-kDA laminin receptor (LR), was discovered in cancer cells [21] and found in normal cells, such as muscle and nerve cells [22], and the fact that different types of LR and many isoforms of laminins have been reported [22–24] to occur in many cancer and normal cells [22–24] have also caused much controversy. Accordingly, a thorough examination of the signal elements through which tea catechins execute their modulations of fat cells and other related cell types should help clarify these observations.

This paper reviews the evidence for the connections between tea, and obesity and diabetes based on various *in vivo* and *in vitro* studies. Since more data on the antiobesity and antidiabetic effects of green tea exist than on black and oolong tea, we focus herein on green tea and green tea catechins more than on fermented teas or catechin byproducts (*i. e.*, theaflavins and thearubigins) that are produced during fermentation [15]. Green tea also contains numerous vitamins and other phytochemicals that are important to human health [8–13], but these are also not discussed in this review. The mechanistic results discussed in this paper may possibly be utilized in the treatment of obesity and diabetes using tea catechins.

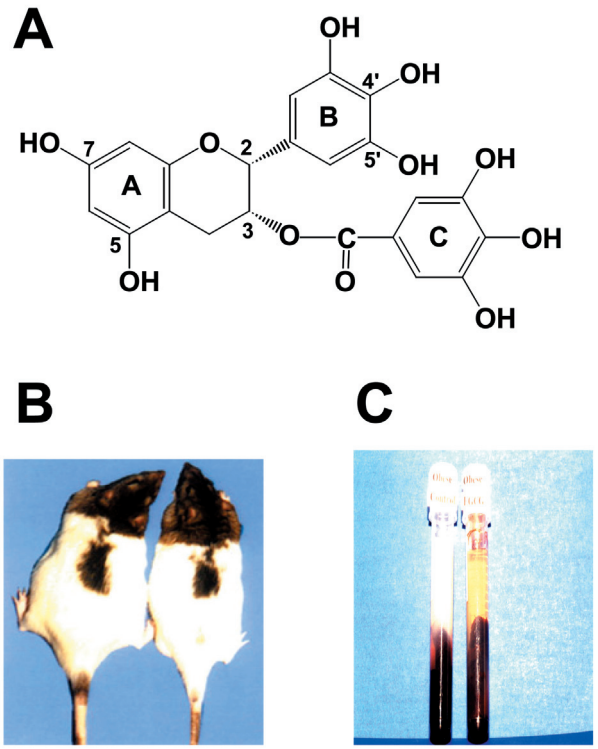


Figure 2. Effects of the intraperitoneal (IP) epigallocatechin gallate (**A**: EGCG, 72–90 mg/kg bw) administration on body weight (**B**) and serum levels of total lipids (**C**), as indicated with the turbidity, of male obese Zucker rats [18]. **B**: *left*, obese Zucker rats (average initial and final weight: 533 ± 12 and 622 ± 16 g) given a daily IP injection with vehicle (0.01 M sterile phosphate buffer solution); *right*, obese Zucker rats (average initial and final weight: 539 ± 12 and 566 ± 14 g) given a daily IP injection of EGCG for 14 days. **C**: *left*, blood was collected from obese Zucker rats (average initial and final weight: 431 ± 14 and 463 ± 14 g) given a daily IP injection with vehicle after 4 days, and then centrifuged at a force of 9400*g* and a temperature of 4°C for 30 min; *right*, blood was collected from obese Zucker rats (average initial and final weight: 434 ± 18 and 417 ± 18 g) given a daily IP injection with EGCG after 4 days, and then centrifuged at a force of 9400*g* and a temperature of 4°C for 30 min.

2 Green tea and obesity

Consumption of green tea beverage is considered by the people of Oriental countries to be beneficial for maintaining a healthy body. However, clear scientific evidence for the antiobesity effect of green tea has not been available until recently. There is some evidence that green tea and its catechins, especially EGCG (Fig. 2A), reduce body weight as well as tissue and blood fat (Table 1, Fig. 2B and C) [18, 19]. The mechanisms of action of green tea and EGCG

Table 1. Effects of (–)-EGCG on body weight, food intake, adipose tissues, adipogenic hormones, and serum nutrients[a)]

Parameters	Effects	Dose/route/duration	Models	References
Body weight	↓	70–92 mg/kg bw, ip, 4–8 days	SDR, LZR, or OZR	[18]
	↓	50 mg/kg bw, ip, 3 days	Mice	[107]
	–	81 mg/kg bw, p.o., 1 wk	SDR	[18]
	–	0.5–1% w/w diet, p.o., 4 wk	Rat	[32]
	–	300 mg/kg bw, p.o., 7 days	Mice	[33]
	↓	1% w/w diet, p.o., 5 months	Mice	[40]
	↓	0.5–1% w/w diet, p.o., 29 days	Mice	[41]
	–	500 mg/kg bw, p.o., 3 days	Mice	[41]
Food intake	↓	70–92 mg/kg bw, ip, 4–8 days	SDR, LZR, or OZR	[18], [19]
	↓	81 mg/kg bw, p.o., 1 wk	SDR	[18]
	–	0.5–1% w/w diet, p.o., 4 wk	Rat	[32]
	–	1% w/w diet, p.o., 5 months	Mice	[40]
	–	0.5–1% w/w diet, p.o., 29 days	Mice	[41]
	–	500 mg/kg bw, p.o., 3 days	Mice	[41]
Subcutaneous adipose tissues	↓	70–92 mg/kg bw, ip, 7–8 days	SDR or LZR	[18]
	–	70–92 mg/kg bw, ip, 4 days	OZR	[18]
	↓	1% w/w diet, p.o., 5 months	Mice	[40]
Abdominal adipose tissues	↓	70–92 mg/kg bw, ip, 4–8 days	LZR or OZR	[18]
Epididymal adipose tissues	–	70–92 mg/kg bw, ip, 4–8 days	SDR, LZR or OZR	[18]
	↓	1% w/w diet, p.o., 5 months	Mice	[40]
	↓	0.5–1% w/w diet, p.o., 29 days	Mice	[41]
Body fat	↓	70–92 mg/kg bw, ip, 1 wk	SDR	[18]
	↓	0.5–1% w/w diet, p.o., 29 days	Mice	[41]
Hormones[b)] Leptin	↓	70–92 mg/kg bw, ip, 4–8 days	SDR, LZR, or OZR	[18]
	↓	81 mg/kg bw, p.o., 1 wk	SDR	[18]
	↓	1% w/w diet, p.o., 5 months	Mice	[40]
	↓	0.5–1% w/w diet, p.o., 29 days	Mice	[41]
Rstn	↓	20–100 μM, 3–6 h	3T3–L1 adipocytes	[94]
Cholesterols	↓	70–92 mg/kg bw, ip, 4–8 days	SDR, LZR, or OZR	[18]
	↓	0.5–1% w/w diet, p.o., 4 wk	Rat	[32]
	↓	500 mg/kg bw, p.o., 7 h	Mice	[33]
Glucose	↓	70–92 mg/kg bw, ip, 4–8 days	SDR, LZR, or OZR	[18]
	↓	1% w/w diet, p.o., 5 months	Mice	[40]
	–	0.5–1% w/w diet, p.o., 4 wk	Rat	[32]
	–	0.5–1% w/w diet, p.o., 29 days	Mice	[41]
Fatty acid	–	70–92 mg/kg bw, ip, 7 days	SDR	[18]
	–	0.5–1% w/w diet, p.o., 29 days	Mice	[41]
Lipid	↓	70–92 mg/kg bw, ip, 4–8 days	SDR, LZR, or OZR	[18]
Protein	–	70–92 mg/kg bw, ip, 4–8 days	SDR, LZR, or OZR	[18]
Triglyceride	↓	70–92 mg/kg bw, ip, 4–8 days	SDR, LZR, or OZR	[18]
	↓	1% w/w diet, p.o., 5 months	Mice	[40]
	↓	0.5–1% w/w diet, p.o., 29 days	Mice	[41]
	–	0.5–1% w/w diet, p.o., 4 wk	Rat	[32]

a) HDL cholesterol levels in rats fed a high cholesterol diet are increased, while LDL cholesterol levels are decreased. Abbreviations: SDR, Sprague–Dawley rat; LZR, lean Zucker rat; OZR, obese Zucker rat; ip, intraperitoneal; p.o., oral; ↑, increased; ↓, decreased; –, no effect.

b) Serum levels of leptin were found to decrease in SDR, LZR, and OZR from Kao *et al*. [18], while leptin mRNA levels were reported to decrease in mice from Wolfram *et al*. [40] and Klaus *et al*. [41]. EGCG was also found to reduce Rstn mRNA and protein expressions in 3T3–L1 adipocytes [94].

involve certain pathways, including (1) the decrease in the energy intake [18, 19], (2) the increase in energy expenditure [25, 26], and (3) the alterations in the activities of fat, liver, muscle, and intestinal cells [8, 18, 19]. However, these mechanisms are dependent on the route of administration, the number of times administered, the duration and dosages of treatments, the purity of the green tea extract or catechins, and the techniques and assay models employed.

2.1 Green tea, body weight, and food intake

Unlike other structurally related catechins, such as (–)-epicatechin (EC), (–)-epicatechin gallate (ECG), and (–)-epigallocatechin (EGC), EGCG given to rats by an intraperitoneal injection at a dosage of 70–92 mg EGCG *per* kg body weight (bw) daily reduced body weight by about 20–30% within 2–7 days (Table 1) [18]. Proximate composition

analysis of animals showed that Sprague–Dawley rats treated with EGCG for 7 days had no change in percent water or protein content, a 50% decrease in carbohydrate content (2.5% in control and 1.3% in the EGCG-treated group), but a 65% reduction in fat content (from 4.1% in the control to 1.4% in the EGCG-treated group) [18]. EGCG treatment decreased subcutaneous fat by 40–70% and abdominal fat by 20–35%, but not epididymal fat, in male Sprague–Dawley and lean Zucker rats. A 20% loss of abdominal fat was seen in obese male Zucker rats within 4 days of EGCG treatment. This suggests the selective effect of EGCG on adipose tissues. Decreased adipose fats may explain the observed decreases in adipose tissue mass and the sequent hypolipidemia of animals treated with EGCG [18, 19].

The effective dose of EGCG on body weight is 30–50 mg EGCG/kg bw. However, rats gradually adapt and within 1 wk higher doses of EGCG (100 mg/kg bw) are needed to reduce or prevent body weight increases [8]. The body weight loss is reversible; when EGCG administration is stopped, animals regain body weight lost. In support of these antiobesity effects of EGCG [17], other *in vivo* data have shown that EGCG or EGCG-containing green tea extract reduces food uptake (Table 1), lipid absorption, and serum lipids, triglyceride, cholesterol, and leptin levels (Table 1), as well as stimulating energy expenditure, fat oxidation, high-density lipoprotein (HDL) levels, and fecal lipid excretion [8, 18, 19]. The loss of appetite might involve neuropeptides other than leptin, since EGCG is effective in reducing the body weight of lean and obese (leptin receptor-negative) female and male rats [18]. However, EGCG did not change plasma levels of neurohormones, such as ACTH, neuropeptide Y, CRF, urocortin, and galanin. But, plasma levels of cholecystokinin (CCK), a satiety hormone secreted from the gastrointestinal systems and brain, are elevated in rats given a diet supplemented with tea polyphenols [27]. Also, EGCG injection causes the increase in plasma TNFα (a cachectic factor) levels [8]. Further study is required to determine whether other hypothalamic and gastrointestinal neuropeptides, such as melanocortins and ghrelin [7], are also responsible for the antiobesity and anorexigenic effect of EGCG in animals [18].

2.2 Green tea and the endocrine system

Effects of green tea on the endocrine system have only very recently been reported. Rats treated with EGCG exhibited significant changes in various endocrine parameters [18]. After 7 days of IP treatment with EGCG at a dosage of 85 mg/kg bw, circulating levels of testosterone were reduced by about 75% in male rats and 17β-estradiol levels by 34% in female rats. Dose-dependent effects of EGCG on levels of serum leptin, IGF-I, insulin, growth hormone (GH), and luteinizing hormone (LH) were also observed. These suggest that low LH, GH, and insulin production leads to reduced serum levels of sex hormones, IGF-I, and leptin, respectively. However, EGCG did not alter serum levels of corticosterone, suggesting the selective effect of EGCG on endocrine systems. Because sex steroids, insulin, and IGF-I are known to be anabolic hormones and because the latter two hormones are responsible for stimulating fat cell growth and differentiation [7], the decreases in plasma insulin and IGF-I levels by EGCG may be related to decreased adipose fats and the subsequent decreases in adipose tissue mass and serum lipid levels in rats [18]. Oral administration of green tea polyphenols in the diet (5%) of male rats for 8 wk showed such endocrinological effects as inducement of goiters, increased plasma thyroid-stimulating hormone (TSH) levels, and decreased body weights and blood tri-iodothyronine (T_3) and thyroxine (T_4) levels, while no significant change in follicle-stimulating hormone levels was observed [28]. Since thyroid hormones are known to stimulate the basal metabolic rate and to be closely involved with weight loss [7], the observed decreases in plasma T_3 and T_4 levels with ingestion of tea polyphenols [28] suggest that other hormones, such as norepinephrine, may be responsible for the increasing thermogenesis and the subsequent weight loss in humans or animals treated with green tea. This assumption is supported by the fact that EGCG ($IC_{50} \approx 0.2$ μM) inhibits the activity of catechol-*O*-methyltransferase (COMT) [29], the metabolizing enzyme of norepinephrine and epinephrine, the fact that green tea increases energy expenditure in obese rats through β-adrenoceptor activation of thermogenesis in brown adipose tissues (BAT) [30], and the fact that green tea catechins enhance inward Ca^{2+} currents and modulate stimulus-secretion coupling in bovine adrenal chromaffin cells [31]. Since the sympathoadrenal system is known to affect brain functions [7], it is possible that EGCG may reduce food intake [18] *via* stimulating adrenaline activity [25, 26].

2.3 Routes of EGCG administration

The antiobesity and anorexigenic effects of EGCG and green tea are dependent on the route of administration (Table 1). While the effects of IP injection of EGCG on body weight loss and food intake have been observed, these effects are not effective after oral administration of EGCG within 7–14 days [18] or even 4 wk [32]. In mice, oral administration of ECG or EGCG (300 mg/kg bw) within 7 days did not alter body weight [33]. This may be due to inefficient absorption of EGCG [30] or its rapid metabolism to inactive molecules in the digestive tract [11] and suggests that the effects of IP injection of EGCG are not caused by interaction of EGCG with food or by EGCG's action within the gastrointestinal tract. An alternative explanation for the difference between IP and oral adminis-

trations is that both types of administrations may provide different EGCG concentration in the blood as discussed in Section 8. Although oral administration of EGCG is not effective within 14 days, long-term oral consumption of EGCG or EGCG-containing green tea extracts may mimic some of the acute EGCG effects caused by IP injection of EGCG and may be beneficial to health. This contention is evidenced by the fact that oral consumption of EGCG or green tea can reduce rat, mice, and human body weight [34–41], lower rat, mice, and human serum cholesterol [42, 43], increase rat HDL cholesterol [32], decrease rat and human low-density lipoprotein (LDL) cholesterol [32, 39, 43], and lower rat, mice, and hamster blood triglyceride [40–48]. Based on oral and IP effects of EGCG on serum hormones and nutrients, long-term consumption of green tea or EGCG appears to influence the incidence of obesity (Table 1) as reported from clinical studies [36, 37].

2.4 Epidemiological observations and clinical studies

Although some epidemiological studies have not provided clear-cut evidence for a link between tea consumption and body weight [43, 49–54], several studies have shown that tea intake is associated with decreased serum concentrations of total cholesterol and lipoprotein cholesterol levels. For example, two Japanese studies have indicated that green tea consumption (>ten cups/day) was inversely associated with serum levels of total cholesterol and LDL cholesterol [43, 49], but not with body weight index, HDL cholesterol, or triglycerides [43, 49]. In another Japanese study with men over 40 years of age, higher levels of green tea consumption (>ten cups) were associated with an increased proportion of HDL cholesterol together with a decreased proportion of low and very LDL cholesterols and serum concentrations of total cholesterol and triglyceride, but not with body weight index [50]. In a Taiwanese study involving 1103 subjects, the habitual tea consumption for more than 10 years was inversely associated with percent body fat and body fat distribution. Unfortunately, this study does not clarify what types of tea were consumed [51].

In a recent clinical study in France, green tea extract containing 25% EGCG exerted its reductions of body weight (4.6%) and waist circumference (4.5%) in moderately obese patients 3 months after treatment [36]. In a Japanese study, the subjects ingesting one bottle oolong tea containing 690 mg green tea catechins *per* day for 12 wk had a lower body weight, body weight index, waist circumference, body fat mass, and subcutaneous fat area than did the subjects ingesting one bottle oolong tea containing 22 mg catechins *per* day [37]. These studies indicate the possible beneficial effects of green tea catechins to reduce human body weight. However, a Netherlandish study [52] concluded that there was no effect of consumption of green tea (six cups/day) on body weight index and plasma lipid and antioxidant levels in normal-weight smokers during a 4-wk period, while plasma cholesterol and LDL cholesterol tended to decrease after consumption of 3.6 g green tea polyphenols *per* day. But, 18 healthy Japanese men were given a green tea extract containing 254 mg catechins [53]. After 1-h administration, their plasma level of EGCG reached 0.27 nM, while plasma phospholipids, total cholesterol, and triglyceride did not change. But, the plasma phosphatidylcholine hydroperoxide level decreased from 74 pM in controls to 45 pM in EGCG-treated subjects, suggesting that tea catechins are effective as antioxidant.

There are also controversial observations in the green tea's regulation of energy expenditure and fat oxidation in humans [25, 54]. In a respiratory chamber study in France, ten healthy men ingested at breakfast, lunch, and dinner with green tea extract that contains 50 mg caffeine and 90 mg EGCG [25]. EGCG-containing green tea extracts that contain caffeine are more potent than caffeine alone at stimulating 24-h energy expenditure and fat oxidation and urinary norepinephrine excretion in humans [25]. However, a Netherlandish study involving 104 overweight and moderately obese male and female subjects, ages 18–60 years and BMI 25–35 kg/m^2, the level of green tea extracts that contain caffeine (104 mg/day) and EGCG (323 mg/day) and are given for 13 wk was not associated with weight maintenance after 7.5% body weight loss in very-low-energy-diet subjects [54]. This study also showed that habitual caffeine consumption affected weight maintenance in the green tea treatment. Together, these clinical observations indicate that long-term (≥3 months), but not short-term, oral consumption of green tea appears to reduce human body weight or fat. The difference in regulating body weight from these studies may be attributable to the protocols employed, the purity of green tea extracts, the period of administration, the percentage of the caffeine in tea, and the physiological condition of the subjects.

3 Molecular and cellular bases for the antiobesity effects of green tea catechins

There are many reports on the molecular and cellular bases for antitumor and antioxidant effects of green tea catechins [8–13]. However, relatively little is known about the underlying mechanism of their action in the regulation of body weight. Fortunately, certain bases, including (1) decreasing digestive enzyme activity, (2) increasing lipolytic activity, (3) decreasing lipogenic activity, (4) increasing fat oxidation and thermogenesis, (5) to modulating the activity and expression of lipoproteins, (6) decreasing the cell numbers of preadipocytes and adipocytes, and (7) decreasing hor-

mone-stimulated proliferation of preadipocytes and their differentiation to adipocytes, have recently begun to be examined and should help explain the *in vivo* antiobesity and hypolipidemic effects of EGCG and green tea on animals and humans.

3.1 Modulation of lipolytic enzymes

Lipases, the rate-limiting step in lipid breakdown in the hydrolysis of triglycerides to fatty acids or 2-monoglyceride, are a diverse family dependent on their substrates and the positions in substrates of the bonds that they hydrolyze [48]. The milieu in which lipases act are heterogeneous: the lipid substrate is dispersed as an emulsion in aqueous medium, or is present as fat droplets, and the enzyme acts at the interface between the lipid and aqueous phases [55]. A green tea extract containing 25% catechins (AR25) and rich in EGCG inhibits gastric lipase (GL) and pancreatic lipase (PL), the enzymes involved in lipid digestion, *in vitro* at 40 and 80 mg tea extract *per* gram of substrate, respectively (Table 2) [56]. The activity of PL is specifically by EGCG with an IC_{50} of 0.34–11 μM [55, 56]. This inhibition is apparently due to a catechin-induced lipid emulsification process since the addition of EGCG (55–1300 μM) not only dose-dependently reduces cholesterol solubility in biliary micelles but also alters the size of the mixed lecithin/taurocholate/cholesterol micelles [58]. This suggests that the reduced lipid emulsification and digestibility may be responsible for lowering intestinal cholesterol absorption, total fat absorption, and serum triglyceride and cholesterol levels. The observed increases of fecal lipids in animals treated with EGCG [32] or green tea catechins [42] support this notion. In addition, an *in vitro* cell study [59] indicates that EGCG, but not (+)-catechin (C), seems to stimulate the activity of hormone-sensitive lipase (HSL), which is within adipocytes and is responsible for lipid mobilization from adipose tissues to other peripheral tissues. This was evident by the fact that EGCG (10–20 μg/mL), but not C, stimulated an increase in glycerol release by 3T3–L1 adipocytes into the cytosol 4 h after incubation with each catechin [59]. In contrast, EGCG, but not C or EC, at 100 μg/mL inhibited adrenaline- and adrenocorticotropic hormone-induced lipolysis in the primary fat cells of rats, as indicated by decreased release of fatty acid [60, 61]. *In vivo*, green tea consumption for 8–16 wk did not alter adrenaline-induced lipolytic activity in rat adipose tissue [62]. It is unknown whether the difference from these studies is attributable to the presence of caffeine in tea or the distinct properties between heterogeneous (primary cells) [60, 61] and homogeneous (secondary cells) [59] populations of fat cells. However, changes in the expression of lipoprotein lipase, a cell surface enzyme that hydrolyzes triglycerides in lipoprotein and is related to the transport of dietary and *de novo* lipids from the blood to tissues, was not observed in obese rats treated with EGCG [40]. Together with these studies, EGCG appears to affect the activity of the various types of lipases in a different manner, and this effect is dependent on the physiological conditions of animals and the presence of the specific type of hormones in the experimental models. The clear-cut evidence for the effects of EGCG on a variety of lipases remains to be determined. Whether the effects of EGCG on a variety of lipases are related to the reduced fatty liver and adipose tissues of animals or human treated with EGCG or green tea [18, 32, 36, 37, 40, 41] also requires more-thorough studies. Because caffeine in green tea has the well-known lipolytic effects on animals and fat cells [23], it should be cautious to explain that the lipolytic effect of whole green tea or green tea extracts used in the experiments is due to the activity of tea catechins or caffeine in the tea.

Table 2. EGCG inhibition of lipid-related enzymes in cell-free systems[a)]

Enzymes	IC_{50}, μM	References
Lipogenic enzymes		
ACC	310	[63]
Aromatase	60	[28]
FAS	52	[64]
Lanosterol 14α-demethylase	>100	[68]
Oxidosqualene:lanosterol cyclase	>100	[68]
Squalene epoxidase	0.7	[68]
Lipolytic enzymes		
GL[b)]	10	[56]
PL	0.34–11	[57, 137]
Oxidoreductase		
Glycyrrhizin-binding lipoxygenase	10	[75]
Lipoxygenase	10	[74]
Type 1 5α-reductase	15	[72]
Type 2 5α-reductase	74	[72]
Others		
COMT	0.2	[29]

a) Activity and expression of some enzymes which have been found to be affected by tea catechins in cell or animal systems include: ACC [40], FAS [65, 66], ME [40], G6PDH [40], G3PDH [40, 67], SCD1 [40], acyl-CoA oxidase [72], medium-chain acyl-CoA dehydrogenase [72], UCP2 [41], UCP3 [41], fatty acid translocase [73], carnitine palmitoyltransferase [40], and HSL [59].

b) The unit is expressed as mg green tea extract *per* gram of tributyrin substrate.

3.2 Inhibition of lipogenic enzymes

Green tea catechins are found to possess antilipogenic activity (Table 2). They inhibit the activity and/or expression of lipogenic enzymes, such as acetyl-CoA carboxylase (ACC), fatty acid synthase (FAS), malic enzyme (ME), glucose-6-phosphate dehydrogenase (G6PDH), glycerol-3-

phosphate dehydrogenase (G3PDH), and stearoyl-CoA desaturase-1 (SCD1) [11–15]. ACC is the rate-limiting step in fatty acid synthesis for catalyzing the conversion of acetyl-CoA to malonyl-CoA (the precursor of fatty acids). When green tea ECG or EGCG was incubated with rat liver ACC, both catechins (with a Ki of 310 μM) inhibited the activity of ACC [63]. However, neither (+)-catechin, EC, nor EGC has that effect. A recent report also showed that EGCG supplementation down-regulates ACC mRNA expression in obese mice [40]. When EGCG was incubated with chicken FAS, the second enzyme to catalyze the conversion of malonyl-CoA to fatty acyl-CoA, it inhibited FAS activity with an IC_{50} value of 52 μM [64]. EGCG's inhibition of FAS activity is composed of reversible fast-binding inhibition and irreversible slow-binding inactivation [64]. The observed inhibition may be explained by the rapid EGCG association with NADPH binding site or adjacent area of β-ketoacyl reductase of FAS [64]. Because FAS shows high levels of activity in LNCaP human prostate cancer cells, EGCG treatment at 100 μM for 24 h inhibited 52% FAS activity [65]. Concurrently, reduced lipids, such as triglycerides, phospholipids, and cholesterol, were also observed. In addition, EGCG is reported to suppress FAS mRNA and protein levels expressed by MCF-7 breast cells, and EGCG signaling may involve the down-regulation of EGF receptor pathway since EGCG inhibits the activity of EGF receptor and its downstream Akt and Sp-1 proteins [66]. Decrease in the expression of hepatic ME and G6PDH, two enzymes that generate NADPH for fatty acid biogenesis, have also been observed in obese mice treated with EGCG [40]. In addition, the activity [67] and expression [40] of G3PDH, the rate-limiting step in triglyceride biosynthesis, are decreased by EGCG treatment. Gene expression of SCD1, the rate-limiting enzyme in the synthesis of monounsaturated fatty acids by liver and adipose tissues, is suppressed in EGCG-treated obese mice [40]. Taken together, EGCG or green tea appears to reduce fatty acid and triglyceride synthesis *via* inhibiting lipogenic enzymes and this may explain the hypolipidic liver, fat cells, and blood.

Several cholesterol-related enzymes can be also regulated by green tea catechins (Table 2), supporting the possible hypocholesterolemic effect of green tea. Squalene epoxidase, the rate-limiting step in cholesterol biogenesis which catalyzes the conversion of squalene to (3S)-2,3-oxidosqualene, is inhibited by green tea catechins [68]. IC_{50} values were found to be 0.69 μM for EGCG, 1.3 μM for ECG, 3.2 μM for EGC, 0.13 μM for theasinensin A (a polymerized catechin), and 73 μM for gallic acid. However, caffeine and EC were inactive. The inhibitory activity may be caused by specific binding to the enzyme or by scavenging reactive oxygen species required for the monooxygenase reaction. At 100 μM, EGCG also inhibited the activity of two other cholesterol-biosynthetic enzymes, lanosterol 14α-demethylase and oxidosqualene:lanosterol cyclase [68]. Together with the inhibition of micelle formation [56] and the stimulation of fecal cholesterol excretion [32, 38] by EGCG as discussed in Section 3.1, EGCG's inhibition of cholesterol-biosynthetic enzymes may be related to the low plasma cholesterol levels observed in rats IP-injected with EGCG [18]. Interestingly, an *in vivo* study [69] shows that no effect of green tea on the activity of HMG-CoA reductase and cholesterol 7α-hydroxylase was observed, suggesting the selective effect of green tea or EGCG on the cholesterol-biosynthetic enzymes. It should be noted that tea extracts and gallated catechins have also been observed to inhibit other steroid-related enzymes, such as 11β-hydroxysteroid dehydrogenase [70], 5α-reductase [71], and aromatase [28]. Decreased 5α-reductase activity may be related to weight loss of androgen-dependent organs, such as prostates and seminal vesicles, observed in EGCG-treated male rats [18]. Decreased aromatase activity may be related to low blood estrogen levels and the subsequent weight loss of estrogen-dependent organs, such as uterus, observed in EGCG-treated female rats [18]. These observations may explain the beneficial use of EGCG in the prevention of prostate and breast cancers in patients [8].

3.3 Stimulation of fat oxidation

There are controversial observations in the green tea's regulation of energy expenditure and fat oxidation in humans [25, 54, 72, 73]. As partially discussed in Section 2.4, this controversy may be attributable to the protocols employed, the purity of green tea extracts, the number of times administered, the period of administration, and the method of administration (capsules *vs.* tea drinking). However, there is evidence for a trend of green tea and EGCG increasing energy expenditures and fat oxidation. First, EGCG-containing green tea extracts that contain caffeine are more potent than caffeine alone at stimulating 24-h energy expenditure and fat oxidation in humans [25] and for stimulating *in vitro* the respiration rate of rat BAT [26]. Second, the *in vitro* prolonged thermogenic effect of a green tea extract on BAT can be mimicked by EGCG [26]. Third, EGCG inhibits COMT activity [29]. Diminished COMT activity delays the metabolism of norepinephrine and epinephrine and may cause subsequent increases in sympathetic thermogenesis. Fourth, green tea reduces body fat accretion and increases energy expenditure in obese rats through β-adrenoceptor activation of thermogenesis in BAT [30]. Finally, although the expression of uncoupling protein-1 (UCP1) is retained in the BAT of EGCG-treated obese mice [41], the increases in the fatty acid oxidation induced by EGCG or tea catechins were observed as evidenced by increased levels of liver acyl-CoA oxidase, medium-chain acyl-CoA dehydrogenase, and UCP2, increased levels of muscle UCP3 and fatty acid translocase, and

Table 3. Effects of (−)-EGCG on receptors in cell-free or cell culture systems[a)]

Receptors	Activity/Expression	Kd or EC50	Models	References
Adhesion receptor: 67-kDa LR	↓ Binding to laminin	Kd = 39.9 nM	*In vitro*, A549	[21]
Cell death receptor: FAS/APO-1 receptor	↑Expression	50–100 μM	HepG2 cell	[112]
Growth factor receptors: EGF receptor	↓ Activity	1.1 μM	Cell-free	[108]
FGF receptor	↓ Activity	2.2 μM	Cell-free	[108]
HER-2/neu	↓Activation	22 μM	YCU-H891	[109]
IGF-I receptor[b)]	↓Activity and expression	44 μM	SW837	[110]
	↓Activity	50 μM	3T3–L1 cell	Kao *et al*. [Unpubl.]
	↑Activity	50 μM	Hep G2 cells	[87]
IGF-II receptor	↓Association with G protein	20 μM	3T3–L1 cell	Kao *et al*. [Unpubl.]
Insulin receptor[b]	↓Activity	50 μM	3T3–L1 cell	Kao *et al*. [Unpubl.]
	↑Activity	50 μM	H4IIE cells	[87]
PDGF receptor	↓Activity	2.3 μM	Cell-free	[108]
VEGF receptor	↓Activation	~13.6 μM	B-CLL cells	[111]
Steroid receptors: Androgen receptor	↓Transcription	10–20 μM	LNCaP	[106]
Estrogen receptor α[c)]	↓Binding for E_2	480 μM	*In vitro*	[107]
	↑Gene expression	28 μM	MCF-7	[107]
Estrogen receptor β[c)]	↓Binding for E_2	97 μM	*In vitro*	[107]
	↑Gene expression	19 μM	MCF-7 cell	[107]
Others: AH receptor	↓Transcription	<50 μM	Hepa cell	[113]
IgE receptor	↓Expression	~50 μM	KU812 cell	[114]
LDL receptor	↑Expression	<10 μM	HepG2, HeLa	[78]
PPARγ	↓Expression	10 μM	3T3–L1 cell	[92]
	↓Expression	100–400 μM	3T3–L1 cell	Kao *et al*. [Unpubl.]
TLR-4	↓Activation	<5 μM	AGS cells	[115]

a) Cell lines: A549, human lung cancer cells; HepG2, hepatocellular carcinoma cells; YCU-H891 cells, human nasopharynx carcinoma; SW837 cells, human colorectal cancer cells; 3T3–L1, fibroblast; H4IIE, rat hepatoma cells; B-CLL cells, B-cell chronic lymphocyte leukemia; LNCaP, human prostate cancer cell; MCF-7 cells, human breast cancer cells; Hepa cell, mouse hepatoma cells; KU812 cells, human basophilic cells; HeLa cells, human cervical adenocarcinoma cells; AGS cells, human gastric cancer cells. Abbreviations: EGF, epidermal growth factor; FGF, fibroblast growth factor; IGF, insulin-like growth factor; PDGF, platelet-derived growth factor; VEGF, vascular epithelial growth factor; AH receptor, aryl hydrocarbon receptor; IgE, immunoglobulin E; LDL, low-density lipoprotein, PPARγ, peroxisome proliferators activated receptor γ; TLR-4, toll-like receptor 4; E_2, 17β-estradiol; ↑, increased; ↓, decreased.

b) Using the immunoprecipitation method, Waltner-Law *et al*. [87] have reported that EGCG increases tyrosine phosphorylation of the insulin receptor and insulin receptor substrate-1 in H4IIE rat hepatoma cells as well as tyrosine phosphorylation of IGF-I receptor in Hep G2 cells when cells are treated with 50 μM EGCG for 30–180 min. This study on liver cells is different from those of SW827 human colorectal cancer cells and 3T3–L1 cells, suggesting the cell line-dependent effect of EGCG on insulin receptor and IGF-I receptor.

c) EGCG was examined for its ability to compete with [^{3}H]-17-estradiol for binding to ERα and ERβ and to elicit reporter gene activity in MCF-7 cells transiently transfected with either chimeric ERα or ERβ [107].

increased muscle β-oxidation [40, 41, 72, 73]. Thus, the EGCG- and green tea-enhanced thermogenesis and fatty acid oxidation may be mediated *via* increasing adrenaline activity and hepatic and muscular β-oxidation and this may support their hypolipidic and antiobese effects.

3.4 Modulation of lipoproteins

Green tea catechins are potent antioxidants and free radical scavengers due to the hydroxyl groups in their chemical structure [8–13]. They dose-dependently inhibit LDL oxidation in endothelial cells induced by reactive oxygen species [8]. Another *in vitro* assay also showed that EGCG and ECG are more active than EC and EGC in preventing LDL oxidation from copper sulfate induction [8]. Lipid peroxidation enzymes, such as lipoxygenase [74], were inhibited by EGCG with an IC_{50} of 10 μM (Table 2). This effect has also been observed in cell culture or in skin. EGCG can also prevent the *in vitro* phosphorylation of glycyrrhizin-binding lipoxygenase by casein kinase II [75]. These observations suggest that the reduction of lipid peroxidation by EGCG and green tea may be related to the mechanism by which they modulate lipid transport in lipoproteins of the lymphatic blood system [76]. It should be mentioned that EGCG is recently found to inhibit radical reaction of apolipoprotein B-100 [77] and increase LDL receptor expression in HepG2 and HeLa cells (Table 3) [78] and this may relate to the mechanism by which EGCG or green tea reduces blood cholesterol in animals [14] and humans [43, 49–52].

3.5 Antimitogenic effect on fat cells

Obesity is characterized by increased number and size of fat cells. Accordingly, a thorough examination of the signal

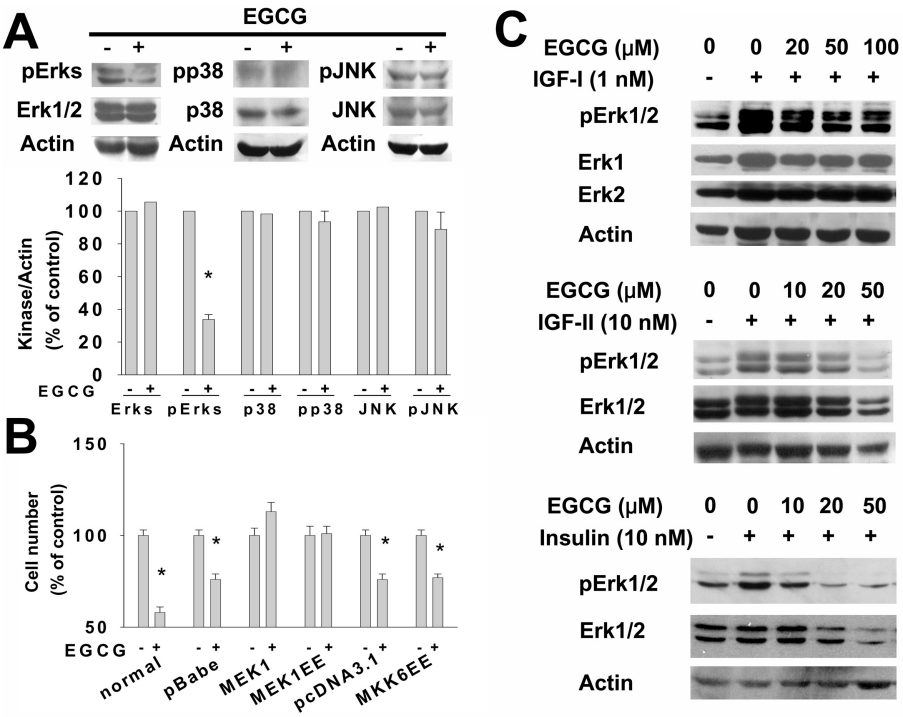


Figure 3. Antimitogenic effect of EGCG on preadipocytes was dependent on MEK1 pathways [68]. (**A**) EGCG at 50 μM for 4 h reduced protein amounts of phospho-Erk-1/2 (pErks), but did not alter protein amounts of p38, phospho-p38 (pp38), JNK, or phospho-JNK (pJNK) in Day 3 3T3–L1 preadipocytes (day 1 being the day of cell inoculum). (**B**) Transient overexpression of MEK1 and its constitutively active MEK1S217E/S221E (MEK1EE) mutant. However, neither MKK6EE nor the vehicles (pBabe and pcDNA3.1) prevented the reduced numbers of 3T3–L1 preadipocytes by 50 μM of EGCG for 48 h. (**C**) Effect of EGCG on IGF-I-, IGF-II-, and insulin-induced stimulation of amounts of phospho-Erk1/2 proteins. Twelve-hour-starved day 3 preadipocytes were pretreated with EGCG (0–100 μM) for 2 h and then stimulated with IGF-I (1 nM), IGF-II (10 nM), or insulin (10 nM). After 1 h of treatment, amounts of phospho-Erk1/2, Erk1/2, and actin proteins were analyzed by Western blot analysis.

element through which EGCG and green tea executes modulation of preadipocyte mitogenesis should help in the prevention and control of the development of obesity. To our knowledge, a clear assessment of the antimitogenic effect of green tea EGCG on 3T3–L1 preadipocytes began recently. Preadipocyte proliferation as indicated by increased numbers of cells [19] and greater incorporation of bromodeoxyuridine [79] was inhibited by EGCG (Fig. 3) in dose-, time-, catechin-, and growth phase-dependent manners. For example, the IC_{50} values of EC, EGC, and ECG in day 3 (days 1–6 with day 1 being the day of cell inoculum) preadipocytes were all >50 μM during the 72 h of treatment except for 10–20 μM EGCG at 24–72 h, while the IC_{50} values of EC, EGC, and ECG in day 2 (latent), and day 6 (confluent) preadipocytes were all >200 μM at 48 h of treatment except for 50–300 μM EGCG. The growth phase-dependent effect of EGCG suggests that different growth stages of preadipocytes have different sensitivities or signals to individual GTCs. The fact that the amounts of phospho-Erk-1 and phospho-Erk-2 in log-phase, but not latent and confluent, preadipocytes were significantly reduced by EGCG supports this contention.

The MAPK family is an essential part of the signal transduction machinery of signal transmissions from cell surface receptors and environmental stimulation, and it contains three major MAPK subfamilies, Erk, p38, and JNK [8, 11, 12]. It has been proposed that they serve as signal elements in several types of cells through which EGCG may regulate cell growth, and they were found to modulate mitogenic and adipogenic signalings of growth factors in 3T3–L1 preadipocytes [80]. Our laboratory observed that acute (4–h) exposure to EGCG induced a decrease in phosphorylated Erk1/2 in 3T3–L1 preadipocytes, but did not alter total levels of MEK1, Erk-1, Erk-2, p38, phospho-p38, JNK, or phospho-JNK (Fig. 3A) [79]. This suggests that EGCG acts on a specific type of MAPK, especially in the Erk MAPK family. This contention is also supported by the evidence that transient amplification of phospho-Erk1/2 content by transfecting MEK1 cDNA or its active mutant cDNA to 3T3–L1 preadipocytes prevented EGCG-induced decreases in their cell number (Fig. 3B) [79]. Total levels of MEK1 protein in vehicle-, MEK1- or MEK1EE-transfected preadipocytes were not affected by any of the EGCG treatments. In contrast, overexpression of either MKK6EE (a constitu-

tively active mutant of MKK6 to activate p38 MAPK kinase) or MEKK1 (an MEKK1 construct favoring the activation of JNK MAPK kinase) did not prevent EGCG-induced decreases in the number of preadipocytes [79]. Taken together, these findings demonstrate that a suppressive effect of EGCG on preadipocyte proliferation is likely mediated *via* Erk MAPK-dependent and p38 MAPK- and JNK MAPK-independent pathways.

In cell-free systems, the inhibition of MAPK activity by EGCG is competitive with the myelin basic protein substrate and is noncompetitive with ATP [81]. In contrast, the activities of certain protein phosphatases were stimulated by 15% by 10–50 μM EGCG [82]. In cultured 3T3–L1 cells, our laboratory found that EGCG (20–50 μM) significantly prevented increases in phosphorylated Erk1/2 by IGF-I (1 nM), IGF-II (10 nM), or insulin (10 nM) (Fig. 3C) [79] and concomitantly reduced activity of IGF-I and insulin receptors, as indicated by a decrease in the phosphotyrosine-IGF-I and phosphotyrosine-insulin receptors, and an association of the IGF-II receptor with Gαi-2 protein (unpublished observations). Further study is required to determine whether the EGCG-induced decrease in phosphorylated Erk1/2 from preadipocytes is mediated *via* the association of Raf with MEK1 or *via* stimulation of phosphatase activity.

The antimitogenic effect of EGCG on preadipocytes also depends on Cdk2 pathway [79] by which EGCG modulates the cell cycle and growth arrest of preadipocytes. This is evidenced by the fact that doses of 20–100 μM EGCG decreased Cdk2 activity at 4, 24, and 48 h and reduced its protein levels at 48 h (Fig. 4), but not at 4 and 24 h [79]. Also, EGCG dose-dependently induced G1 growth arrest at 24 and 48 h after treatment. In addition, increased Cdk2 activity *via* the transfection of $Cdk2^{+/+}$ cDNA to preadipocytes prevented EGCG-induced decreases in their Cdk2 activity and cell number and EGCG-induced increases of G1 arrest, while decreased Cdk2 activity *via* the transfection of dnCdk2 cDNA to preadipocytes reduced the 5–day growth of preadipocytes and increased their G1 growth arrest. These observations suggest that the effect of EGCG in inducing preadipocyte antimitogenesis and growth arrest is dependent on a Cdk2 pathway and requires inactivation by the Cdk2 protein. Since cyclin D1 is a G1 cyclin associated with Cdk4 and Cdk6 proteins which favor cell cycle arrest at the G1 checkpoint [23], decreased cyclin D1 protein expression by EGCG for 24–48 h suggests the possibility of Cdk4- and Cdk6-related effects of EGCG on preadipocyte growth arrest. However, 20 μM EGCG treatment for 48 h did not affect total levels of Cdc2 protein, and that 100 μM EGCG treatment for 48 h reduced levels of Cdc2 protein less than of Cdk2 (Fig. 4B). Accordingly, EGCG appears to act on a specific type of Cdks in preadipocytes, but further studies are required to illustrate this contention.

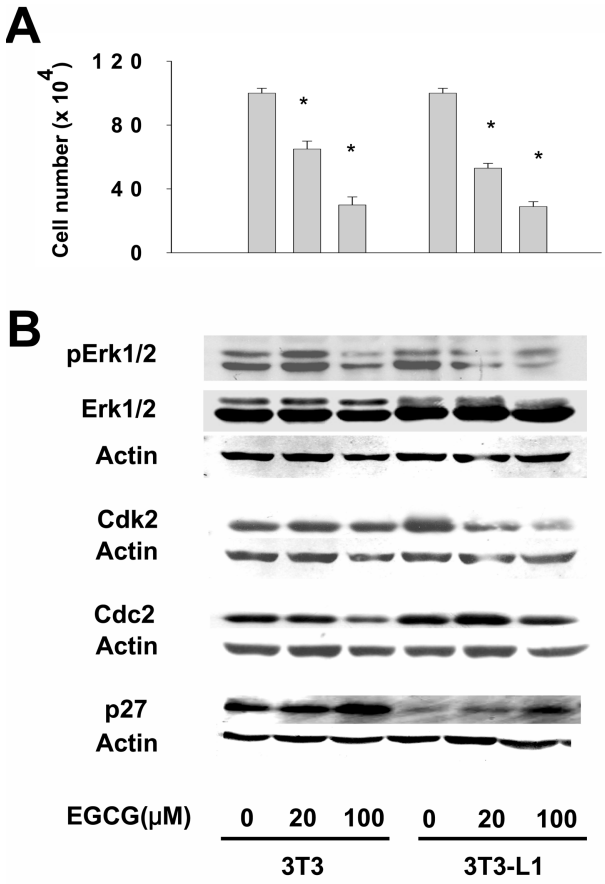


Figure 4. Differential effects of EGCG on altering the cell number (**A**) and levels of phospho-Erk1/2 (pErk1/2), Erk1/2, Cdk2, Cdc2, and p27 (**B**) proteins of Day 3 3T3–L1 preadipocytes and 3T3 fibroblasts (day 1 being the day of cell inoculum) observed after 48 h of treatment [68]. Numbers of cells were determined by the trypan blue exclusion method. Amounts of Erk1/2 (Erk1 and Erk2) and phospho-Erk1/2 (pErk1/2 or pErks) proteins were measured by Western blots and then expressed after normalization to actin. Data are expressed as the mean ± SEM from triplicate determinations. $*p < 0.05$ *versus* the control for a given type of cell line.

Regulation of Cdk2 activity of cultured cells occurs at multiple levels, involving the synthesis of subunits and the association of inhibitory proteins such as p21 and p27 [23]. On these bases, a decrease in the Cdk2 activity of 3T3–L1 preadipocytes induced by 4 h of EGCG treatment may result from the observed increase in the association of Cdk2 with p21 and p27 by EGCG [79]. But, the short-term effect of EGCG in reducing Cdk2 activity should be unrelated to the availabilities of p21, p27, and Cdk2 because EGCG did not alter their protein levels in this period. However, increased levels of p21 and p27, but not of p18 or Cdk2, observed with the 24-h EGCG treatment may be responsible for the increased association of Cdk2 with p21 and p27, but not p18, by EGCG, thereby leading to low Cdk2 activity and a subsequent rise in the percent of G0/G1 arrest. In addition,

decreased levels of Cdk2 protein and increased levels of p21 and p27, but not p18, observed with the 48-h EGCG treatment may explain the EGCG-induced increase in the association of Cdk2 with p21 and p27 (Fig. 4B), but not p18, thereby resulting in a decrease in Cdk2 activity and an increase in the percentage of cells undergoing G0/G1 arrest. These results suggest that EGCG may act on a particular type of preadipocyte in the CKI family to reduce Cdk2 activity.

Green tea catechins have numerous biological activities that can possibly provide various health benefits [8–13]. In most but not all cases, gallated catechins, especially EGCG, are more active than other catechins. This contention is supported by our findings in 3T3–L1 preadipocytes that at the same dose and duration of treatment, EGCG was generally more effective than EC, ECG, and EGC in changing the number of cells, the amount of incorporated BrdU, percentages of the four phases of the cell cycle, activities of MEK1 and Cdk2, and levels of Cdk2, cyclin D1, and CKIs [79]. The observed catechin-specific effects of green tea suggest that EGCG may act differently from EC, EGC, and ECG in regulating preadipocyte growth. According to the nature of the unique structures of these catechins tested [8, 15], EGCG contains the largest number of hydroxyl groups on its three aromatic rings among the tea catechins, and these hydroxyl groups may be important for hydrogen bonding. Also EGCG has both gallyl and galloyl groups, which have some conformational flexibility, that may also be important for interactions with other molecules. Further exploration of the chemical basis of the antimitogenic activity by EGCG on preadipocytes is needed to understand differences in the mechanism of EGCG's action compared to those of EC, EGC, and ECG on these processes.

Mechanistic studies of green tea EGCG have reported its cell type-dependent manner [8, 11, 12]. This may be explained by the fact that the sensitivity of different normal, transformed, and cancer cell lines to green tea EGCG varies [8], although such differences may be due to the cell culture techniques and assay methods employed. Our laboratory used the same experimental culture condition and assay methods to look at whether a cell type-specific effect of EGCG occurs [79]. We observed that the IC_{50} value of EGCG for reducing the cell number was lower in 3T3–L1 cells than in 3T3 fibroblast (Fig. 4) and human KB oral cancer cells, supporting the existence of different sensitivities of these cell lines to EGCG, as similarly reviewed by Liao *et al*. [8]. Such differences between 3T3 fibroblasts and 3T3–L1 preadipocytes induced by EGCG may be explained by observations that the decrease in phosphorylated Erk1/2 of the former cells in response to the 48-h EGCG treatment was much less than that in the latter cells, and that levels of Cdc2 and Cdk2 proteins were, respectively, decreased and increased by EGCG in the former cells, while Cdc2 levels in the latter cells were decreased much less by EGCG than were Cdk2 levels [79]. Although 3T3–L1 cells are subcloned from 3T3 fibroblast [83], these observations [79] suggest that EGCG may work differently in the two cell lines in modulating mitogenesis *via* altering different phases of their cell cycles and/or the extent of apoptosis, and that the Cdk2 transducer may play different roles with EGCG in the two cell lines.

3.6 Apoptotic effect on fat cells

The resulting decrease in the number of preadipocytes by EGCG could be attributable to its inhibition of cell mitogenesis as discussed in Section 3.5, but also to its induction of preadipocyte apoptosis [84]. This is evident by the observations that EGCG at doses of 100–400 μM reduced the cell viability of preadipocytes by 15–30%, induced the appearance of DNA fragmentation, and increased the activity of the caspase-3 protein, an apoptotic enzyme. At the same dose and duration of treatment, EGCG was generally more effective than EC, ECG, and EGC in changing these apoptotic parameters of preadipocytes, suggesting that these catechins may act differently in regulating preadipocyte death. The resulting decreases in the cell viability may be attributable to the formation of DNA fragments. Fragmented DNA by EGCG may be caused by its increase in caspase-3 activity, since this enzyme which exhibited significant induced activity increases by 100–400 μM, but not 20 μM, EGCG is a downstream apoptotic enzyme able to activate DNase [23]. When the inhibitor of caspase-3, Z-VAD-FMK, was used, it stopped the increase in caspase-3 activity by EGCG [84]. Taken together, these observations suggest that activation of the caspase-3 protein may be related to the mechanism by which EGCG carries out its apoptotic effect on preadipocytes. Interestingly, a contradictory observation is that EGCG inhibited the activity of human caspase-3 in an *in vitro* cell-free system [84], suggesting that the effect of EGCG in altering caspase-3 activity might be species-specific, or that an increase in caspase-3 activity induced by EGCG in the cell culture system might be indirectly mediated through activation of upstream caspase-3 activators.

Other various antiapoptotic and apoptotic factors, including Bcl2, Bad, Bax, and p53, have been reported (23), and some of them can be regulated by EGCG [8]. More studies are required to determine whether any of them are responsible for the apoptotic effect of EGCG on preadipocytes. However, the induction of preadipocyte apoptosis by EGCG was also dependent on Cdk2 and is likely mediated by inactivation of the Cdk2 protein [84]. This is based on the observations that inactivation of the Cdk2 protein *via* transfection of dnCdk2 cDNA to 3T3–L1 preadipocytes caused the formation of DNA fragments, and that overexpression of Cdk2

via the transfection of $Cdk2^{+/+}$ cDNA to 3T3–L1 preadipocytes prevented 48-h decreases in Cdk2 activity and protein levels and formation of DNA fragments by 100 μM EGCG. Unfortunately, the Cdk2 pathway required for the mechanism of the apoptotic action of EGCG is still not clear. However, the possibility still remains that decreases in Cdk2 expression and activity of 3T3–L1 preadipocytes by EGCG may link to the activation of caspase-3 *via* changing the mitochondrial transmembrane potential, cytochrome *c* release, and the caspase-9 activity (an activator of caspase-3) as reported for the apoptotic effect of the tea polyphenol, theasinensin A (an EGCG dimmer), in human lymphoma cells [85].

While green tea catechins are excellent antioxidants that act as radical scavengers and protect cell components from radical damage, these antioxidants can also be prooxidants under certain conditions [8]. This contention is also supported by the reported dose-dependent effect of EGCG on neuroblastoma cells [17, 86], in which at 50 μM of EGCG-induced oxidative stress and radical formation and up-regulated proapoptotic genes, while at 20 μM it acted as an antioxidant and up-regulated antiapoptotic genes. In parallel to this, EGCG at 50 μM altered glucose metabolism in rat hepatoma cells *via* modifying the redox state of the cell [87]. Despite the rise in reactive oxygen species, the cell viability of hepatoma was not adversely affected by 1 mM EGCG. Through the use of trypan blue dye exclusion method, we found that concentrations of EGCG below 50 μM, the cell viability of day 3 preadipocytes remained at 90–100% during the 48-h treatment, while doses of 100–400 μM EGCG decreased the cell viability of preadipocytes by 15–30% and induced their apoptosis [84]. When the number of viable cells in proliferation or cytotoxicity assays was examined by a CellTiter 96^{R} Aqueous One Solution Reagent (Promega, Madison) containing a tetrazolium compound, the cell viability of day 3 preadipocytes decreased by 10, 20, 25, 35, and 50%, respectively, after 10, 20, 50, 100, and 400 μM of EGCG treatment. Taken together, at 100–400 μM, EGCG may have some cytotoxic effects on 3T3–L1 preadipocytes, and this may be attributable to its prooxidant activity as reported for hepatoma [87] and neuroblastoma cells [17, 86].

A recent study showed no significant effects of EGCG on either viability or apoptosis of preconfluent preadipocytes when cells were treated with 50–200 μM within 24 h and then examined with MTS and terminal deoxynucleotidyl transferase dUTP nick-end labeling (TUNEL) methods, respectively [88]. The difference of this study [88] from our study [84] may be attributable to the sensitivity of the different stages of preadipocytes [79], the duration of EGCG treatment, or the analytical methods exploited. However, Lin *et al*. [88] have reported that the 24-h treatment of 200 μM EGCG resulted in the apoptotic effect on mature 3T3–L1 adipocytes when cells were examined by the TUNEL assay. Unfortunately, this study did not examine the mechanism by which EGCG exerted its apoptotic effects on adipocytes.

3.7 Modulation of fat cell adipogenesis and differentiation

3T3–L1, a murine preadipose cell line first established by Green and Kehinde [83] from one of the subclones of mouse embryonic fibroblast line 3T3, is a useful experimental model for examining the mechanism of action of a nutrient and hormone in the growth, differentiation, development, and metabolism of the white adipose tissues (WAT). The reasons for this are as follows. First, 3T3–L1 cells are nutrient and hormone sensitive [83, 89, 90]. Second, they can lead to the formation of a fat pad when cultured preadipocytes are transplanted under the skin of athymic mice [91]. Third, they can be like other established cell lines to be propagated indefinitely in serial culture before the confluent, but they can be like normal fat cells to be growth-arrested by contact inhibition when cells are confluent [89, 90]. Fourth, they can differentiate homogeneously (>90%) from preadipocytes to adipocytes under a culture medium with the mitotic and adipogenic inducers, such as insulin, dexamethasone (Dex), and 1-methyl-3-isobutylxanthine (MIX) [83, 89, 90]. The growth after the confluence is called the mitotic clonal expansion, but it can be stopped in another growth arrest, so-called postmitotic growth arrest "G_D" [90]. G_D-arrested cells are characterized by their ability to differentiate in the absence of DNA synthesis and by their sensitivity to the mitogenic effect of MIX [90]. Then, cells enter the growth arrest that is irreversible and they are considered to be terminally differentiated, which is marked by the massive triglyceride accumulation because of the increased lipogenic enzyme activity [90]. The amounts of triglyceride can be stained by oil red O and quantitatively read the absorbance at a wavelength of 540 nm [83]. Finally, differentiated adipocytes have a similar nutritional or hormonal control scheme of lipid metabolism to that of WAT *in vivo* [90].

The regulation of fat cell differentiation by EGCG and green tea began to be published in 2000 when 3T3–L1 adipocytes were examined [19]. EGCG (10 μM), but not EC, EGC, or ECG, inhibited insulin-induced increases in cell number by 34% and the triglyceride content by 54% during a 9-day period of differentiation (Fig. 5) [19]. This suggests the possible catechin-specific effect of green tea on preadipocyte clonal expansion and/or adipogenic differentiation. EGCG at a dose of 10–100 μM also reduced the cell number and triglyceride content of differentiating preadipocytes treated with Dex, MIX, and insulin [19]. As discussed in Sections 3.2 and 3.5, EGCG was shown to be a lipogenic

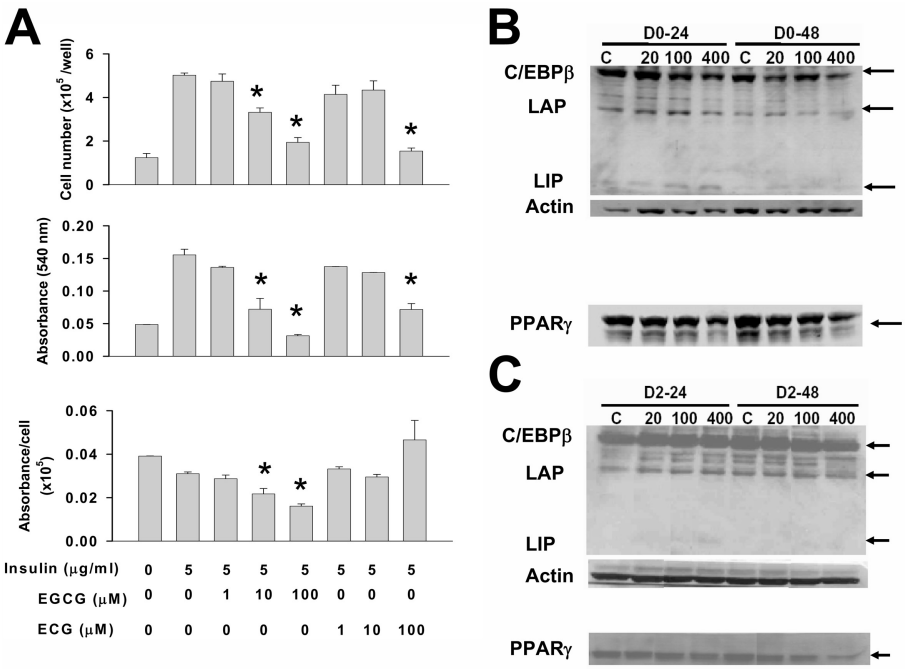


Figure 5. Effects of green tea ECG and EGCG on differentiation of 3T3–L1 preadipocytes (unpublished observations). **A**, Two-day postconfluent preadipocytes (designated with *day 0* being the day of cells added with Dex and MIX) were treated with insulin (**A**) in the presence or absence of ECG or EGCG during a 9-day period of differentiation. (**B**) and (**C**), Two-day postconfluent preadipocytes treated with insulin, Dex, and MIX in the presence or absence of EGCG (20–400 μM) for 24 and 48 h when EGCG was added on either *day* 0 (**B**; designated with D0) or *day* 2 (**C**; designated with D2). *$p < 0.05$, insulin *versus* insulin + ECG or insulin *versus* insulin + EGCG. ECG, (−)-epicatechin gallate; EGCG, (−)-epigallocatechin gallate. In (**A**), adipocyte differentiation was determined as indicated by triglyceride accumulation which was stained by oil red O and the absorbance was read at a wavelength of 540 nm (A540). Content of triglyceride was normalized to the change in the number of differentiating cells as indicated by absorbance *per* cell. In (**B**) and (**C**), total cell lysates (~50 μg protein) were measured by Western blot analysis to determine adipogenic differentiation factors, such as C/EBPβ and PPARγ, with their individual antibodies. C/EBPβ is translated from multiple in frame AUG sites giving rise to proteins of 35, 32, and 20 kDa. Liver inhibitory protein (LIP; p20C/EBPβ) functions as a dominant-negative inhibitor of liver activator protein (LAP; p32C/EBPβ).

inhibitor and it inhibited insulin-, IGF-I- and IGF-II-stimulated cell proliferation of 3T3–L1 preadipocytes through reducing the Erk phosphorylation levels. Therefore, the *in vitro* effect of EGCG on fat tissues may be mediated by modulation of hormone-stimulated cell proliferation and differentiation or by inhibition of fat cell functions. Further study by our laboratory using the Western blot analysis showed that EGCG reduces levels of adipogenesis-related transcriptional factors, such as C/EBPβ and PPARγ, of differentiating 3T3–L1 preadipocytes treated either with Dex, MIX, and insulin (Fig. 5B and C) or with MIX only (unpublished observations). The effect is dose- and time-dependent. Similar changes that EGCG at 10–30 μM reduces levels of PPARγ2 and C/EBPβ and amounts of lipid accumulation (~25–50% decrease) in differentiating 3T3–L1 adipocytes have been reported [92]. In this study, CG, EGC, and EGCG suppressed intracellular lipid accumulation and G3PDH activity in a dose-dependent manner during the differentiation. Together, these observations support the *in vivo* findings that green tea suppresses the expression of PPARγ and the activation of sterol regulatory element binding protein-1 in adipose tissue, and with the *in vitro* observations that EGCG at 50–100 μM inhibits differentiation of C3H10T1/2 mouse mesenchymal cells into adipocytes treated with Dex, MIX, and insulin [40, 45, 92]. In support of these effects of EGCG, Lin *et al.* [88] showed that EGCG at 50–200 μM could reduce the size and number of lipid droplets in differentiating 3T3–L1 adipocytes when it was added with the induction medium from days 0–6 of adipogenesis [88].

3.8 Regulation of resistin (Rstn) gene expression

Rstn is known as an adipocyte-specific secretory hormone that can cause insulin resistance and decrease adipocyte differentiation [93]. Thus, it has been proposed as a biomarker of insulin resistance or adipose tissue mass. As discussed in Sections 2–5, green tea catechins, especially EGCG, have been reported as a chemopreventative agent that blocks

excessive body weight. Whether EGCG exerts its effects through the control of Rstn's production is unknown. Using 3T3–L1 adipocytes, we found that EGCG suppressed Rstn mRNA and protein expression in dose- and time-dependent manners (Table 1) [94]. The IC_{50} of EGCG was ≈100 μM after 3 h. Neither actinomycin D (a transcriptional inhibitor) nor cycloheximide (a translational inhibitor) prevented EGCG-suppressed Rstn mRNA levels, suggesting that EGCG's effect does not require new mRNA and protein synthesis. EGCG did not affect amounts of Erk1/2, phospho-JNK, phospho-p38, and phospho-Akt proteins, but reduced amounts of phospho-Erk1/2 proteins. Overexpression with MEK1 blocked EGCG-inhibited Rstn mRNA expression. These data suggest that EGCG down-regulates Rstn expression *via* a pathway by which can be related to the MEK1-dependent pathways. In addition, the inhibitory effects of other tea catechins on Rstn mRNA expression were observed. As Rstn is known to cause insulin resistance and inhibit adipocyte differentiation, changes induced by EGCG may be related to the mechanism by which EGCG modulates body weight and diabetes that is discussed in Sections 4 and 5.

3.9 An EGCG receptor

Green tea catechins, such as EGCG, have been reported to possess multiple biological functions based on the various laboratory studies. Accordingly, a thorough examination of the EGCG receptor would help determine the primary target of EGCG and elucidate the mechanism of actions of EGCG. The 67-kDa LR, an accessory integrin protein first isolated from cancer cells [95, 96], was found to be an EGCG receptor linking between EGCG and cancer [21]. In particular, physiological concentrations of 0.1–1 μM EGCG can inhibit the growth of LR-transfected lung cancer cells, while the growth-suppressive effect of EGCG was completely blocked by the pretreatment of cells with LR antibody [21]. The Kd value representing the binding affinity of EGCG to LR is about 40 nM when examined in equilibrium binding experiments by surface plasmon resonance and purified recombinant LR protein [21]. EGCG can compete with laminin for binding of LR [21]. Moreover, recent reports showed that EGCG signaling in reducing growth of HeLa cervical cancer cells was mediated *via* the disruption of stress fibers and the contractile ring by 67-kDa LR that reduced the phosphorylation of myosin regulatory light chain [97]. The 67-kDa LR was also reported in association with a lipid raft of the membrane to mediate the suppressive effect of EGCG on IgE receptor in B-cells [98]. This suggests the possible interaction relationship between the EGCG receptor and other types of receptor in a particular type of cells. Because lipid rafts are known to contain specific kinases that are well-known enzymes enabling to generate second messengers within the cell by catalyzing the phosphorylation of specific substances [23], the association of the EGCG receptor with a lipid raft may explain the findings that EGCG exhibits marked effects on kinase activity (*i.e.*, receptor tyrosine kinases, Erk) and the subsequent selective phosphorylation of downstream proteins (*i.e.*, myosin regulatory light chain) as reported in many studies [8–13, 79, 98].

Despite of these attentions, the 67-kDa LR is not only found on cancer cells, but normal cells, such as muscle cells, macrophages, neutrophils, endothelial cells, epithelial cells, hepatocytes, interstitial cells, and neuronal cells also have proteins in this size range that bind laminin [22]. This observation suggests that the 67-kDa LR may also play as an EGCG receptor to regulate the effects of EGCG on these normal cells or other types of cells. Basically, LR, a heterodimeric structure similar to that of receptors for other extracellular matrix proteins such as fibronectin [22, 23], is a member of the integrin family of cell adhesion receptors, and occurs in many types on the membrane of normal cells [22, 23], such as integrin α5 and α6 in murine adipocytes [99]. It is evident that certain types of LRs and laminins are present in preadipocytes or adipocytes and relate to fat cell activity. First, the RT-PCR and the immunological analysis indicated that laminin-8 consisting of α4β1γ1 appears to be the specific isoform of laminin synthesized in adipocytes [24]. Northern blot analysis showed that the mRNA levels of each subunit increased 2.5-fold during differentiation of 3T3–L1 preadipocytes into adipocytes [24]. A different study showed that laminin became degraded during the differentiation process and localized at the surface of spherical cells [100]. Alterations in laminin during differentiation of adipocytes suggest the possible significant changes in the LR at the same time. Second, microarray analysis of differentiation-specific gene expression during 3T3–L1 adipogenesis [101, 102] contains the repressed expression of laminin β2 gene and the induced expression of integrin α6 gene, which has been reported to be colocalized with the 67-kDa LR in cancer cells [22]. In a parallel, 3T3–L1 cells can produce laminin complex which has a size significantly smaller than that of parietal endoderm-like F9 cells [103]. Third, integrin α1β1 can mediate preadipocyte adhesion and migration on laminin-1 [104], and integrin α6 is critically involved in clustering growth-arrested preadipocytes on basement membrane Matrigel [99]. Finally, the high levels of 67-kDa laminin-binding protein occur in mouse 3T3 fibroblast [105] that can be subcloned into 3T3–L1 preadipocytes [83]. Taken together, EGCG appears to regulate growth, apoptosis, and differentiation of fat cells *via* modulating the activity and expression of the adhesion receptor (Table 3). But, this needs further demonstrations. Whether any of these types of LR [22, 23] really serves as an EGCG receptor to be responsible for the significant effects of EGCG on these processes of fat cells also requires further explorations.

3.10 Effects of EGCG on other receptors

Activity of fat cells can be regulated by their membrane and nuclear receptors [23]. Thus, changes in the activation and expression of these receptors induced by EGCG or green tea may be related to the mechanism by which EGCG exerts its effects on fat cells or other types of cells that regulate adipocyte functions. As indicated in Table 3 and as partially discussed in Sections 3.4, 3.7, and 3.9 [21, 78, 92, 106–115], EGCG can alter the activities or expressions of other membrane and nuclear receptors in addition to the 67-kDa LR. For example, EGCG can displace the binding of estrogen receptor α and β to 17β-estradiol *in vitro*, and elicit the Gal4-hERαdef and Gal4-mERβdef-mediated reporter gene expression in MCF-7 cells that are cotransfected with a Gal4-regulated luciferase reporter gene expression [107]. Also, EGCG was found to down-regulate the expression of the androgen receptor in LNCaP prostate cancer cells [106]. It should be noted that MCF-7 and LNCaP cancer cells are fat-synthesizing cell lines. In addition, EGCG can directly or indirectly inhibit the activation or gene expression of most of growth factor receptor, such as EGF, FGF, HER/neu, IGF-I, PDGF, and VEGF receptors, in different cells, while it can stimulate the apoptotic receptor FAS/APO-1 in liver cancer cells [108–112]. In contrast, Waltner-Law *et al*. [87] used the immunoprecipitation method to show that EGCG increased tyrosine phosphorylation of the insulin receptor and insulin receptor substrate-1 in H4IIE rat hepatoma cells as well as tyrosine phosphorylation of IGF-I receptor in Hep G2 cells when cells were treated with 50 μM EGCG for 30–180 min. The study on rat liver cells [87] is different from those observed in SW827 human colorectal cancer cells [110] and 3T3–L1 cells [79], suggesting the cell type-dependent effect of EGCG on insulin receptor and IGF-I receptor. Because some of these receptor components listed as above are known to occur in adipocytes and other types of cells (*i.e.*, liver cells) that can regulate adipose tissues [101, 102], the possibility still remains that EGCG most likely operates its specific effect on the target cells *via* an interaction of the EGCG receptor with any of the particular receptors as reported the suppressive effect of EGCG receptor on IgE receptor in B-cells by catalyzing the phosphorylation of specific substances (*i.e.*, myosin regulatory light chain) [114].

4 Green tea and diabetes

The effects of green tea on diabetes have received increasing attention as data have accumulated. In EGCG-treated, but not ECG-treated, male Sprague–Dawley rats, serum levels of protein, fatty acids, and glycerol were not altered, but significant reductions of up to 32% in serum glucose (Table 1) were observed [18]. Similar changes in these serum nutrients induced by EGCG were observed in male lean and (genetically induced) obese Zucker rats and in mice on high-fat diet [18]. Low levels of plasma glucose are known to reduce oxidative stress and osmotic pressure due to glucose in the cells which thereby improves diabetic complications, such as vascular disease, retinopathy, nephropathy, and neuropathy, as well as the subsequent long-term morbidity and mortality in people with type 1 and 2 diabetes [7]. Accordingly, reductions of oxidative stress-induced DNA breakage, glycation end-products, inflammation, and lipid oxidation by green tea or EGCG should help the possible curative and preventative effects against diabetic complications by green tea.

Vascular disease in rats with streptozotocin (STZ)- or cisplatin-induced diabetes is evidenced by an increased prostaglandin I_2:thromboxane A_2 ratio and by increased phospholipase A_2 and cyclooxygenase activities, and the ratio and enzyme activities are normalized after green tea administration [13]. Nephropathy in these diabetic rats is also improved by green tea through an increased glomerular filtration rate and catalase activity and through decreased thromboxane A_2 production [13]. Hepatotoxicity, fatty liver, and neurodegenerative disease are observed in oxidative stress-induced diabetes, and they are found to be prevented by daily ingestion of green tea or EGCG [13, 17]. These observations support the reductive effects of green tea catechins on oxidative stress-induced diabetes as reported by Sabu *et al*. [116]. In addition, green tea given daily at 1.25% in the drinking water to rats with STZ-induced diabetes for 3 months inhibited diabetic cataracts by lowering plasma and lens glucose levels, cataract rating, the glycation of plasma and lens proteins, lens sorbitol levels, and lipid peroxidation of the plasma and lens [117]. Finally, green tea lowered blood glucose levels in the genetically diabetic *db/db* mice 2–6 h after administration at 300 mg/kg bw, whereas no effect was observed in control mice [118]. Serum proteomic patterns in diabetic mice were also observed to have changed after green tea consumption [118], suggesting that a certain serum protein may be involved in the antihyperglycermic effects of green tea.

The mechanism of the *in vivo* antihyperglycemic action of green tea and EGCG is likely a result of complex events (Fig. 6). For example, the ability of green tea EGCG to reduce blood levels of glucose in rats may be dependent on changes in appetite [18]. Second, EGCG or green tea extract reduces carbohydrate absorption from the intestine of rats with a saccharide-supplemented diet *via* inhibition of the activity of intestinal α-amylase, sucrase, or α-glucosidase [119]. Third, green tea EGCG ameliorates decreases in islet mass induced by multiple low doses of STZ in mice [120]. Fourth, green tea polyphenols reduce oxidative stress in rats with alloxan-induced diabetes as evidenced by decreased

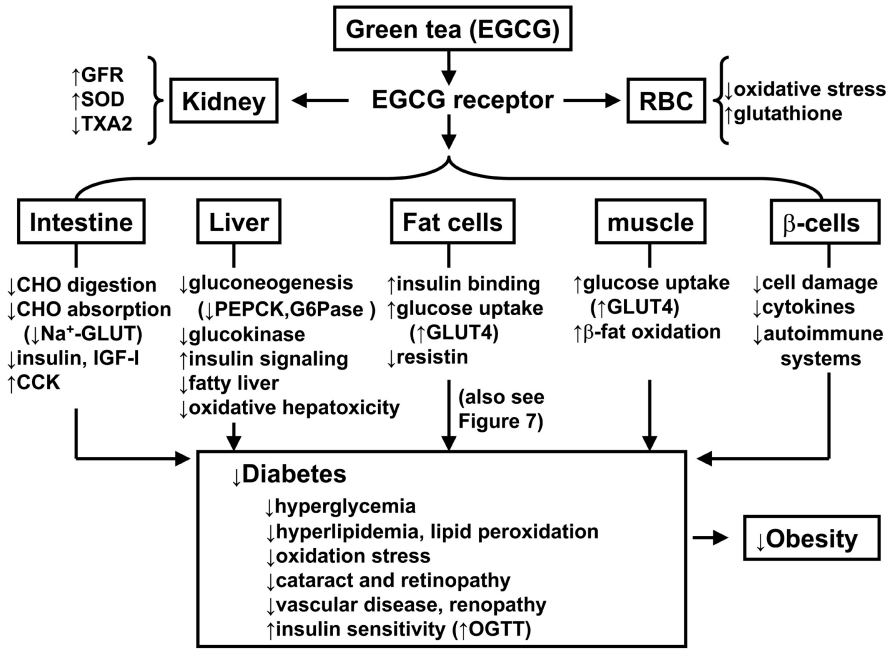


Figure 6. A proposed mechanism of the action of green tea EGCG on diabetes. Signaling of EGCG in its modulation of diabetes is mediated *via* decreases in energy intake and oxidative stress, and stimulation in energy expenditure, all of which are dependent on the activity of fat cells as well as intestine, liver, muscle, kidney, nerve (not shown), and red blood cells.

hepatic ALP, GPT, and LPO and by decreased renal BUN and creatinine levels [116]. Fifth, as type I diabetes can be caused by autoimmune systems that destruct β-cells [7], the observed changes in the immune systems induced by green tea catechins [8] may be related to the mechanism by which they operate their effects on diabetes. For example, when the effect of green tea catechins on immunoglobulin (Ig) production by mesenteric lymph node lymphocytes of male rats is examined, EGCG, ECG, and EGC enhance IgE production at 1 mM, but inhibit it at 100 μM or below [8]. EC does not affect IgE production. All catechins tested exert inhibitory effects on the production of IgA and IgG at 10 μM. EGCG enhance IgA production at 0.1 μM. These results indicate that green tea catechins appear to exert bifunctional effects on Ig production. Moreover, different studies have shown that EGCG exhibits marked anticomplement activities with IC_{50} values of about 10 μM [8]. Sixth, green tea enhances insulin sensitivity of normal [121] and fructose-fed [122] rats as evidenced by increased glucose uptake by muscle cells [45], insulin binding to adipocytes [121, 122], and expression of glucose transporter 4 (GLUT4) by muscle cells [45]. Thus, green tea improves glucose tolerance [121, 122]. Finally, administration of EGCG alone or EGCG-containing green tea extract downregulates the levels of mRNA for gluconeogenic enzymes, such as phosphoenolpyruvate carboxykinase (PEPCK) and glucose-6-phosphatase (G6Pase), in the mouse liver [123]. These *in vivo* actions of EGCG may be partially explained by *in vitro* findings as discussed in Section 5.

5 Molecular and cellular bases for the antidiabetic effect of green tea catechins

While too few molecular and cellular data exist to present a comprehensive mechanism for antidiabetic actions of green tea catechins, some trends are emerging. The mechanism involves certain pathways (Fig. 6) as evidenced by (1) decreased digestive enzyme activity, (2) decreased intestinal GLUT activity, (3) decreased expression of gluconeogenic genes, (4) increased insulin sensitivity, (5) increased insulin-like activity, (6) an altered redox status, (7) increased protections of liver, β-pancreatic, and other cells, and (8) decreased adipose mass and Rstn and blood lipids (discussed in Sections 2 and 3).

5.1 Modulation of carbohydrate-related enzymes and transporters

Green tea catechins are known to regulate the activity and/or expression of various enzymes or proteins related to carbohydrate digestion, absorption, transport, synthesis, and metabolism [8]. *In vitro*, tea catechins can inhibit carbohydrate digestive enzymes, such as salivary α-amylase, intestinal sucrase, α-glucosidase, and gastric H^+,K^+-ATPase [124–126]. Using a rat everted sac, Kobayashi *et al*. [127] showed that green tea polyphenols, especially ECG and EGCG, inhibited the sodium-dependent GLUT (Na^+-GLUT) of intestinal epithelial cells. They further used rab-

bit intestinal brush-border membrane vesicles to demonstrate that 1 mM of ECG or EGCG reduced the glucose uptake by 53 and 35%, respectively, whereas no effect was observed after C and EGC treatments. Although the concentration used in the experiment was very high, the inhibitory activity of tea polyphenols on Na^+-GLUT may have been responsible for the reduction in intestinal glucose absorption observed *in vivo* studies [119]. Taken together, these observations suggest that the reduced digestibility may result in a reduced rate of glucose production in the intestinal tract and subsequent low blood glucose and insulin levels [18, 119].

Aldose reductase catalyzes the conversion of aldoses to sugar alcohol. It is a key enzyme in the synthesis of polyol sugars that cause diabetic complications [7]. As reported by Murata *et al*. [128], EC and ECG are active and EGCG is weakly active, while EGC is not active. The IC_{50} for ECG is 38 μM. This *in vitro* fact [128] may be responsible for the curative effect of green tea catechins on diabetic complications of animals treated with alloxan [116] and STZ [120].

An important signal of EGCG in modulating the glucose production began to be assessed in 2002 when H4IIE rat hepatoma cells were used [87]. Like insulin, EGCG (25–50 μM) increases the activity of the insulin receptor and insulin receptor substrate-1, and it reduces PEPCK and G6Pase gene expressions in a PI3K-dependent manner. Glucose production is also decreased by EGCG. The reductive effect of EGCG on hepatic gluconeogenic enzymes is consistent with those *in vivo* observations reported by Koyama *et al*. [123]. *In vitro* cellular studies have also indicated that EGCG increases PI3K, MAPK, and $p70^{s6k}$ activity [87]. However, pretreatment of *N*-acetylcysteine and superoxide dismutase for 30 min can completely reverse the antigluconeogenic effect of EGCG, but not insulin, in H4IIE cells. This suggests that EGCG differs from insulin in that this catechin regulates these gluconeogenic genes by modifying the redox state of hepatic cells [87].

5.2 Modulation of insulin sensitivity

Green tea appears to enhance insulin sensitivity *in vitro*. Using an epididymal fat cell assay, Anderson and Polansky [129] demonstrated that green tea EGCG induced 17-fold greater insulin activity (as measured with [U-^{14}C] glucose uptake and $^{14}CO_2$ production) than the control. This is consistent with the findings that green tea polyphenols at 0.075% concentrations stimulate insulin (1 nM)-induced glucose uptake by rat adipocytes [121, 122]. In contrast, green tea reduces glucose uptake (20%) and GLUT4 translocation in adipose tissues of male Wistar rats [45] and in 3T3–L1 adipocytes [130]. The controversy may be due to the different periods of green tea administration and the assay systems employed. However, green tea stimulates glucose uptake by skeletal muscle, enhances muscular β-oxidation, and increases GLUT4 translocation, but not protein expression, from the cytosol to the membrane of muscle tissue [45]. Because muscle tissues are more predominant in mass than adipose tissues, the EGCG-induced increases in glucose uptake by muscle cells may explain its *in vivo* hypoglycemic and possible hypolipidemic effects [18]. Despite of the importance of EGCG and green tea, its signaling pathway in modulating insulin sensitivity of these cell types is still unknown.

5.3 Protection of diabetes-related cells

Because of their antioxidant [8–12], green tea and EGCG are able to protect the normal cells against oxidative stress- and cytokine-induced damage and diabetes. *In vitro*, EGCG treatment effectively protects against interleukin-1β (IL-1β)- and interferon γ (IFN γ)-mediated cytotoxicity in RINm5F insulinoma cells (a pancreatic β-cell line) since EGCG reduces the production of nitric oxide, a destructive factor, by these pancreatic cells [131]. This is consistent with the *in vivo* finding [120]. Because the activation of NF-κB by two cytokines is known to regulate the expression of nitric oxide synthase gene [7], EGCG's inhibition of the translocation of NF-κB from the cytosol to the nucleus of pancreatic cells [131] may be responsible for the protective effect of EGCG. When normal and type 2 diabetic erythrocytes are isolated and then treated with tert-butylhydroperoxide (*t*-BHP), an oxidative reagent, in the presence and absence of green tea catechins, the increase in malondialdehyde and decrease in glutathione by *t*-BHP are prevented by tea catechin treatment [132]. EGCG is generally more effective than ECG, EGC, and EC. Also, these catechins modulate the impairment of sodium pump and Na/H exchanger in erythrocytes from NIDDM patients [133]. In nerve cells, the neuroprotective action of EGCG is reported to be involved in the regulation of protein kinase C activity, cell survival, and cell cycle genes [17, 134]. In liver cells, hepatotoxicity and fatty liver (as discussed in Section 3.2) can be prevented by green tea and EGCG [13]. Thus, these observations may explain how EGCG and green tea prevent oxidative stress-induced diabetes *in vivo*.

6 Oolong tea, obesity, and diabetes

Oolong tea is a semifermented tea containing more caffeine and a greater variety of catechins than green tea [15]. It is known to possess antiobesity effect based on a variety of laboratory studies. Oolong tea extract given to mice with high-fat diet-induced obesity at a dosage of 5% reduced their body weight by about 10% within 10 wk, while food intake did not change [135]. In addition, decreases in hepatic triglyceride content (−57%) and decreases in paramedtrial adipose tissue weight (−51%) were observed [136]. Similar

findings for the antiobesity and hypolipidemic effects of oolong tea (1% w/v) on rats with a high-sucrose diet were also observed after 1 month of treatment [136]. However, the later study observed a reduction in food intake due to oolong tea administration [136]. The different effects on food uptake [135, 136] may have been due to distinct periods of administration and amounts of tea polyphenols in the oolong tea extract. In support of these antiobese effects, oolong tea flavan-3-ols (*i.e.*, ECG and EGCG), proanthocyanidins, oolonghomobisflavans, theasinensins, theaflavins, and a few of hydrolysable tannins inhibited the activity of PL, while caffeine had no effect [137]. Results of this *in vitro* study [137] can possibly be translated into oolong tea-reduced fat digestion in animals and humans [135, 136, 138]. In human studies, oolong tea solution (6 g/L water) given for 1 month increased plasma adiponectin levels and LDL particle size and decreased total cholesterol levels in patients with coronary artery disease [138]. Because adiponectin is known to stimulate fatty acid oxidization and improve insulin sensitivity [139], the increase in adiponectin levels induced by oolong tea [138] may be the primary factor in the antiobesity [136, 137], hypolipidemic [135, 136], and hypoglycemic [140] effects of oolong tea. The antiobesity effect is also evident by the fact that oolong tea increases the metabolic rate and fat oxidation in men [141]. Although the increases in energy expenditure and fat oxidation by short-term consumption of oolong tea may be mainly attributable to the presence of caffeine [141], the possibility still remains that long-term consumption of oolong tea may cause its antiobese effect *via* the mechanisms of action of polyphenolic catechins as discussed under green tea.

Oolong tea appears to have an antihyperglycemic effect based on a human study. When the subjects who have type 2 diabetes daily consumed oolong tea (15 g *per* 1500 mL) daily for 30 days, their plasma glucose and fructosamine levels, respectively, decreased from 229 ± 54 to 162 ± 30 mg/dL and from 410 ± 96 to 323 ± 56 μM, whereas those of the water control group did not change [140]. The mechanism of the antihyperglycemic effect of oolong tea is not clear. But, the antidiabetic effect of oolong tea may be similar to that of green tea discussed in Section 3, and may result from reduced carbohydrate digestibility, as evidenced by reduced digestive enzyme activity *in vitro* [137], and from the improved insulin sensitivity, as evidenced by increased insulin-stimulated glucose uptake by adipocytes *in vitro* [129].

7 Black tea, obesity, and diabetes

Black tea is known as a fermented tea which possesses more catechin condensation products, such as theaflavins and thearubigins, and much less EGCG than green tea [15]. It accounts for 80% of the world's tea production. But, the beneficial medicinal properties of black tea have only begun to be assessed in the past 20 years. It was found that rats fed a high-cholesterol diet supplemented daily with black tea extract (100–200 mg/kg bw) by oral drinking showed no difference in body weight or food uptake from the control group [142]. However, black tea treatment reduced serum cholesterol levels, including free cholesterol and LDL-cholesterol, and increased serum HDL-cholesterol levels from the high-cholesterol rats and hamsters [47, 136, 142, 143]. Similar changes in these serum lipids induced by black tea were observed in male Sprague–Dawley rats [35]. In line with these observations, epidemiological evidence from the SU.VI.MAX study showed that increased tea drinking was significantly associated with significantly lower levels of serum triglyceride [144]. In contrast, Bingham *et al*. [145] reported that black tea drinking did not alter blood levels of total cholesterol and triglycerides, LDL-cholesterol, or HDL-cholesterol. Thus, more-thorough studies are required to clarify these controversial reports on the antiobesity and hypolipidemic effects of black tea.

Black tea appears to have an antihyperglycemic effect. First, black tea extract given daily by intragastric administration at a dose of 4 g tea solids/kg bw to rats with STZ-induced diabetes showed reduced plasma glucose levels within 3 wk [146]. It also had the curative and preventive effects [146]. In support of this antidiabetic effect, black tea given daily at 1.25% in the drinking water to rats with STZ-induced diabetes for 3 months inhibited diabetic cataracts by lowering plasma and lens glucose levels, the glycation of plasma and lens proteins, lens sorbitol levels, and lipid peroxidation of the plasma and lens [117]. Second, epidemiological evidence shows that increased tea drinking is associated with significantly lower levels of serum glucose [144, 147]. The mechanism of the antihyperglycemic action of black tea remains to be determined. However, an *in vitro* study reported by Kreydiyyeh *et al*. [148] showed that tea extracts from London tea (a black tea) inhibited intestinal GLUTs, indicating that they may be useful for diabetic patients by reducing glucose absorption. Another explanation is that black tea enhances insulin sensitivity as evidenced by increased insulin stimulation of glucose uptake by rat epididymal fat cells [129]. The activity of black tea is present in the fractions containing EGCG, tannins, theaflavins, and other undefined compounds and absent in the fractions containing caffeine, catechin, and EC. Obviously, further study is required to determine whether any of these compounds is responsible for the *in vivo* hypoglycemic effect of black tea.

8 Bioavailability of green tea catechins

Green tea catechins have numerous biological effects *in vitro*, and generally effects are observed in the range of 10–

100 μM [8]. *In vivo*, plasma concentrations of green tea EGCG or other catechins as generally reported in animals and humans are about 1 μM [8]. However, the absorption and distribution of administrated EGCG and other tea catechins are poor and dependent on catechin structure, purity, dosage, the route of administration, and the tissue involved. For example, after consumption of 1.5 g of decaffeinated green tea solids, the catechins in human plasma reached peak levels in 1.5–2.5 h [149]. At that time, the plasma levels (free and conjugated) of EGCG, EGC, and EC levels were 0.26, 0.48, and 0.19 μM, respectively, while ECG was not detected [149]. Consumption of a single high dose of green tea, equivalent to six cups of tea, can raise plasma levels of catechin to 2–4 μM in 60 min [150]. In a Phase I pharmacokinetic study to determine the systemic availability of green tea EGCG after single oral dose administration of 800 mg EGCG (~eight cups of green tea at once) to healthy subjects, the average maximum plasma concentration values, time to reach maximum plasma concentration, and terminal elimination half-life are about 0.96 μM, 4, and 2 h, respectively [151]. A few minutes after two to three cups of green tea are consumed, the saliva levels of various catechins reach peaks of 39–144 μM EGCG, 11–48 μM EGCG, and 7–28 μM EC [152]. Sixty minutes after intragastric administration of EGCG at a dose of 500 mg/kg bw to rats, the levels of EGCG were 10 μM in plasma, 48 μM in the liver, 0.5 μM in the brain, 565 μM in the small intestinal mucosa, and 68 μM in the colon mucosa [153]. When [^{3}H]EGCG is administered directly into the stomach of mice, radioactivity is found in the digestive tract, liver, lung, pancreas, mammary gland, skin, brain, kidney, uterus, ovary, and testes [154]. When green tea catechins are given by intravenous injections to rats for 5 h, less than 12% of EGCG remains in plasma [155]. When EGCG was administrated at a dose of 100 mg/kg bw to rats by intraperitoneal injection, the plasma concentrations of unmetabolized EGCG, determined by HPLC, were 24, 2, 1, and 1 μM at 0.5, 1, 2, and 24 h, respectively [18]. When EGCG is topically applied in a hydrophilic ointment to mouse and human skin, 1–20% of the dose of EGCG can be absorbed [156]. Together, although the chemical nature of the radioactivity in these tissues was not determined, the evidence as indicated with the existence of EGCG in blood and with the wide-distributed radioactivity of EGCG in many tissues suggests its systemic and numerous effects [8–13]. The wide distribution of EGCG receptor [21–23] supports this contention.

Unfortunately, what is not clear at this time is whether effective doses of catechins can be achieved in adipose tissues simply by consuming tea infusions. Accordingly, the doses of EGCG (the effective dose of most studies is in the range of 10–100 μM) or other tea catechins generally discussed as above compatible with the goal of helping regulating the initiation and progression of obesity and diabetes are a little bit higher, but may be acceptable for the physiological effect of EGCG in animals. Undoubtedly, some of studies are due to the pharmacological effect of EGCG. The observation that EGCG at 1300 μM reduces cholesterol solubility in biliary micelles and alteration of the size of mixed lecithin/taurocholate/cholesterol micelles *in vitro* [58], and the observation that GTE-containing 327 μM EGCG enhanced glucose uptake in rat adipocytes *in vitro* [121] may support this notion. It should be noted that the median lethal dose of a green tea extract containing 85% EGCG and given orally to rats is about 3–5 g/kg [157], and that the lethal doses of green tea polyphenols (SUNPHENON®, Taiyo Kagaku, 60% polyphenol) were estimated to be 3 and 5 g/kg for female and male mice, respectively [27]. In a human study, single oral consumption of 800 mg EGCG or Polyphenon E (EGCG:EGC:EC ratio of 20:3.7:3.1) at once caused some subjects to experience mild headache and fatigue [151]. This report explains that these adverse effects of tea catechins may be related to the study products or have been consequences of the procedures and restrictions that subjects encountered during the course of experiment [151].

9 Conclusions

Tea has historically been used as a folk remedy in both Oriental and Western countries. However, recent evidence based on modern molecular and cellular evaluations of green, oolong, and black tea appears to support possible values of their catechins as medicines for modulating body weight and diabetes. Tea or EGCG appears to primarily affect appetite or energy absorption from the digestive tract, so that it makes an organism to burn fat [18]. However, a few reports indicate that green tea or EGCG can increase fat oxidation and reduce adipose tissue mass without altering the food uptake [25, 40, 41]. Clearly, the functions of tea polyphenols operate through many different mechanisms (Figs. 6, 7). These mechanisms interact to alter the energy balance, the redox status, and activities of obesity- and diabetes-related cells. Despite numerous studies in recent years, our knowledge of the biological activities of oolong and black teas is still very limited.

The different forms of LR, being a tea catechin receptor [21], are known to localize either in a particular type of cells or in many types of cancer and normal cells [22, 23], and this has restricted our understanding of the exact mechanism of actions of tea catechins, particularly EGCG, on body weight and diabetes. However, it is fortunate that an EGCG receptor is identified [21], and its widespread localization in the cells with many isoforms may explain numerous biological effects of EGCG as summarized in Figs. 6, 7. This is also supported by the detectable EGCG concentration in

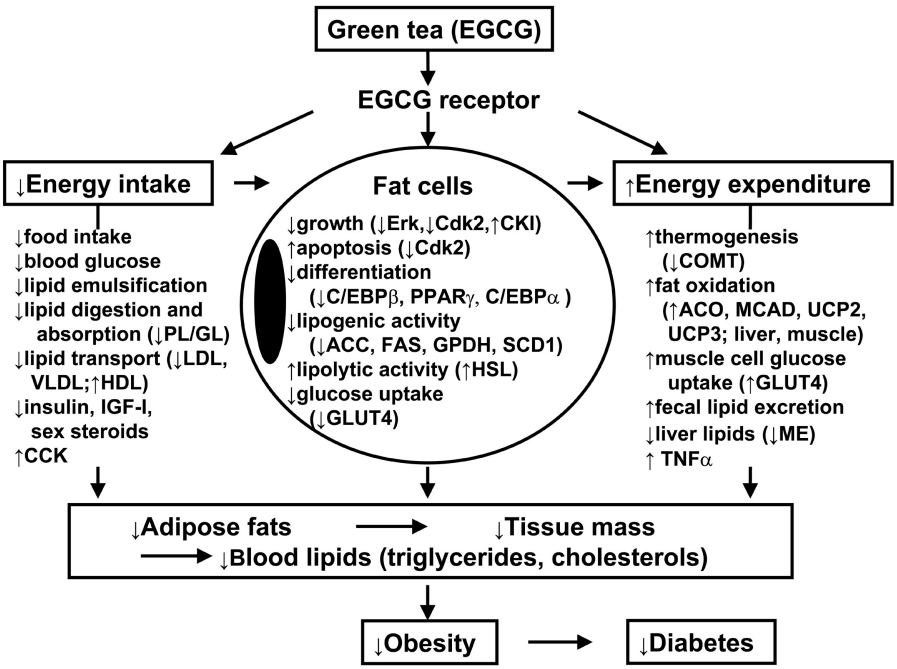
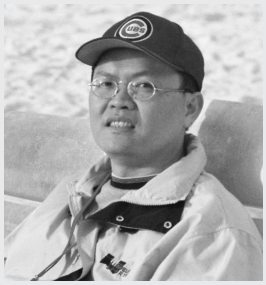


Figure 7. A proposed mechanism of the action of green tea EGCG on obesity. Signaling of EGCG in its modulation of body weight is mediated *via* a decrease in energy intake and stimulation of energy expenditure, both of which are dependent on the activity of fat cells as well as intestine, liver, and muscle cells.

plasma and in many types of cells of animals and humans given intraperitoneally or orally with EGCG or tea catechins [8, 18, 149–156]. An interaction of the EGCG receptor with the specific type of receptor (*i.e.*, growth factor and IgE receptors) that is localized in a particular cell type may suggest a significant effect of EGCG on its target cells. Further in-depth studies of catechin receptors as well as their signaling in different types of cells should help clarify the specific effect of EGCG in obesity and diabetes and thereby enable better utilization of one of the oldest medicines in use today.

Many benefits of tea have received great attention, but it is also necessary to consider the adverse effects that may accompany heavy use of tea or catechins. For example, high doses of EGCG are reported to act as prooxidants, which can lead to normal cell apoptosis [8, 17]. Thus, a further formulation of EGCG or green tea polyphenols designed to improve their low bioavailability *in vivo* is required to reduce their administered amounts by which experimental models and clinical subjects keep the beneficial effects of EGCG and tea polyphenols away from these adverse effects. Several studies using whole tea or tea extract to show the beneficial effects of green, oolong, and black teas suggest that other ingredients of tea should be also considered in making the inclusions of the formulation. Taken together, the mechanistic results of this review may possibly be utilized in the treatment of obesity, diabetes, and other related diseases using tea- and EGCG-based folk medicines.



Yung-Hsi Kao is an assistant professor in the Department of Life Science at National Central University, Taiwan. I received my Ph. D. in Zoology from North Dakota State University of USA in 1997 under the direction of Mark Sheridan. I subsequently did postdoctoral work with Shutsung Liao in the Ben May Institute at University of Chicago. My research focuses on the mechanisms that green tea regulates obesity and diabetes. I am a member of The Endocrine Society and The American Physiological Society.

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10 References

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