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Mini Review

Proposed mechanisms of (−)-epigallocatechin-3-gallate for anti-obesity

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Abstract

Green tea catechins (GTCs) are polyphenolic flavonoids formerly called vitamin P. GTCs, especially (−)-epigallocatechin-3-gallate (EGCG), lower the incidence of cancers, collagen-induced arthritis, oxidative stress-induced neurodegenerative diseases, and streptozotocin-induced diabetes. Also, inhibition of adipogenesis by green tea and green tea extract has been demonstrated in cell lines, animal models, and humans. The obesity-preventive effects of green tea and its main constituent EGCG are widely supported by results from epidemiological, cell culture, animal, and clinical studies in the last decade. Studies with adipocyte cell lines and animal models have demonstrated that EGCG inhibits extracellular signal-related kinases (ERK), activates AMP-activated protein kinase (AMPK), modulates adipocyte marker proteins, and down-regulates lipogenic enzymes as well as other potential targets. Also, the catechin components of green tea have been shown to possess anti-carcinogenic properties possibly related to their anti-oxidant activity. In addition, it was shown that dietary supplementation with EGCG could potentially contribute to nutritional strategies for the prevention and treatment of type 2 diabetes mellitus. In this review, the biological activities and multiple mechanisms of EGCG in cell lines, animal models, and clinical observations are explained.

Keywords: (−)-Epigallocatechin-3-gallate; Anti-adipogenic mechanism; Clinical application

Contents

Abbreviations: EGCG, (−)-epigallocatechin gallate; AMPK, AMP-activated protein kinase; TGs, triacylglycerols; HDL, high density lipoprotein; CDK, cyclin-dependent kinase; ERK, extracellular signal-related kinase; INK, c-Jun-N-terminal kinase; MEK, mitogen-activated protein kinase kinase; Rstn, resistin; Ros, reactive oxygen species; ACC, acetyl-CoA carboxylase; FAS, fatty acid synthase; ME, malic enzyme; G6PDH, glucose-6-phosphate dehydrogenase; G3PDH, glyceol-3-phosphate dehydrogenase; SCD-1, stearoyl-CoA desaturase-1; PPARγ, peroxisome proliferator-activated receptor gamma; C/EBPα, CCAAT/enhancer-binding protein alpha; aP2, adipocyte-specific fatty acid binding protein 2; LDL, low density lipoprotein; GTE, green tea extract; GO, glucose oxidase

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1. Introduction

1.1. Adipocytes and obesity

Adipocytes play a central role in maintaining lipid homeostasis and energy balance in vertebrates by storing triacylglycerols (TGs) or releasing free fatty acids (FFAs) in response to changes in energy demands [1]. However, obesity is a major risk factor for a number of disorders, including diabetes, hypertension, and heart disease [2,3]. For these reasons, estimates of the economic costs, prevalence, morbidity, and mortality associated with being overweight and obese are becoming more important [2,4,5]. The development of obesity is characterized by an increased number of fat cells and their lipids due to the processes of so-called mitogenesis and differentiation, which are regulated by genetic, endocrine, metabolic, neurological, pharmacological, environmental, and nutritional factors [4–6]. Accordingly, an understanding of the mechanism through which a particular nutrient affects the mitogenesis of preadipocytes and their differentiation to adipocytes would help to prevent the initiation and progression of obesity and its associated diseases in humans [6].

1.2. Green tea catechins

Green tea is a widely consumed beverage and has been thought to possess significant health-promoting effects [7–11]. *In vivo*, green tea catechins (GTCs), especially (−)-epigallocatechin-3-gallate (EGCG), lower the incidence of cancers [9–12], collagen-induced arthritis [13], oxidative stress-induced neurodegenerative diseases [14], and streptozotocin-induced diabetes [15]. Also, EGCG can reduce body weight and body fat [16]. In support of this anti-obesity effect of EGCG, other *in vivo* data have shown that EGCG or EGCG-containing green tea extract reduces food uptake, lipid absorption, and blood TGs, cholesterol, and leptin levels as well as stimulating energy expenditure, fat oxidation, high density lipoprotein (HDL) levels, and fecal lipid excretion [8,16]. These *in vivo* observations may be explained by *in vitro* findings that EGCG and caffeine synergistically interact with norepinephrine to stimulate the thermogenesis of brown adipose tissue [17]; that EGCG regulates various enzymes related to lipid anabolism and catabolism, such as acetyl-CoA carboxylase, fatty acid synthase, pancreatic lipase, gastric lipase, and lipoxygenase [8,18]; that EGCG is a potent pro-oxidant and anti-oxidant [19,20]; that EGCG reduces serum- or insulin-induced increases in cell numbers and TG content during a 9-day period of differentiation [20,21]. These *in vivo* and *in vitro* observations suggest that green tea EGCG appears to modulate the mitogenic, endocrine, and metabolic functions of fat cells.

1.3. Proposed anti-obesity mechanisms effected by green tea EGCG

Tea has historically been used as a folk remedy in both East Asian and Western countries [7,8]. Recent evidence based on molecular and cellular evaluations of green, oolong, and black tea appears to support the possible value of their catechins as medicines for modulating body weight and diabetes [8–10]. The functions of tea polyphenols operate through many different mechanisms, and these mechanisms interact to alter the energy balance, the redox status, and the activities of obesity-related cells. As shown in Fig. 1 and Table 1, it is fortunate that an EGCG receptor has been identified [22], and its widespread localization in the cells with many

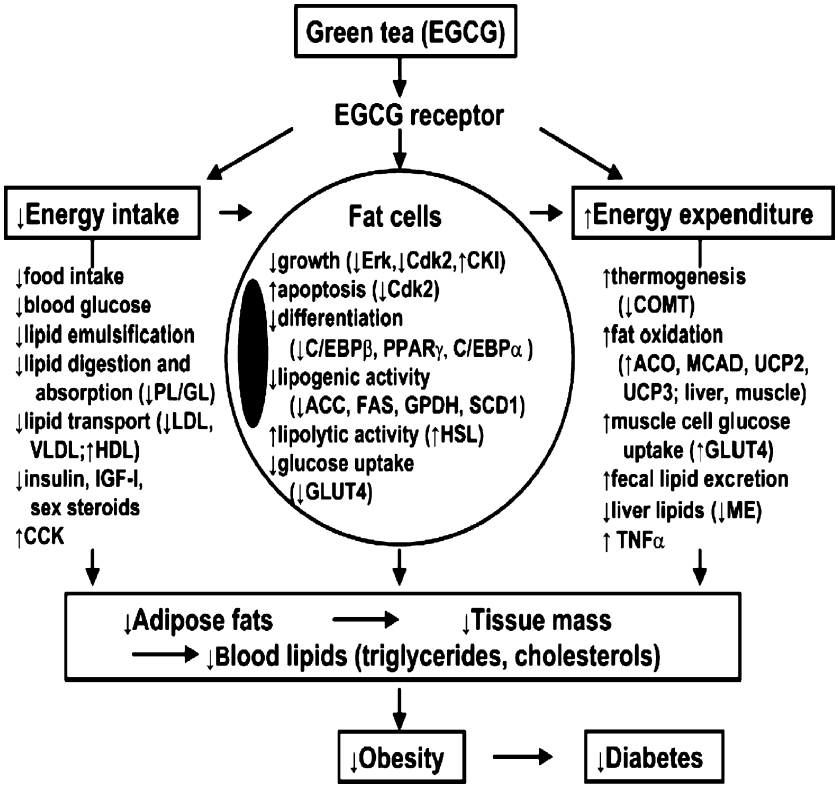


Fig. 1. A proposed mechanism of the action of green tea EGCG on obesity. Signaling of EGCG in its modulation of body weight is mediated via a decrease in energy intake and stimulation of energy expenditure, both of which are dependent on the activity of fat cells as well as intestine, liver, and muscle cells (Ref. [22]).

isoforms may explain the numerous biological effects of EGCG. This is also supported by the detectable EGCG concentrations in plasma and in many types of animal and human cells when EGCG or tea catechins are given intraperitoneally or orally. An interaction of EGCG with the specific type of receptor that is localized in a particular cell type may suggest a significant effect of EGCG on its target cells. Accordingly, a thorough examination of the EGCG receptor would help determine the primary target of EGCG and elucidate the mechanisms of action of EGCG. The 67-kDa LR and accessory integrin protein were first isolated from cancer cells [23,24]. In particular, physiological concentrations of 0.1–1 μM EGCG were shown to inhibit the growth of LR-transfected lung cancer cells, while the growth-suppressing effect of EGCG was completely blocked by the pre-treatment of cells with LR antibody [25]. Moreover, recent reports have shown that EGCG signaling in growth reduction of HeLa cervical cancer cells was mediated via the disruption of stress fibers and contractile rings by the 67-kDa LR that reduced the phosphorylation of the myosin regulatory light chain [26]. Also, the 67-kDa LR was reported in association with a lipid raft of the membrane to mediate the suppressive effect of EGCG on the IgE receptor in B-cells [27]. These results suggested the possible interactive 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 the generation of second messengers within the cell by catalyzing the phosphorylation of specific substances [27], the association of the EGCG receptor with a lipid raft may explain the findings that EGCG exhibits marked effects on kinase activity and the subsequent selective phosphorylation of downstream proteins, as reported in many studies [24,25]. Despite these attentions, the 67-kDa LR is not only found on cancer cells, but normal cells, such as muscle cells, macrophages, neutrophils, hepatocytes, and epithelial cells also have proteins in this size range that bind laminin [28]. This observation suggests that the 67-kDa LR may also play a role as an EGCG receptor to regulate the effects of EGCG on these normal cells or other types of cells. Further in-depth studies of catechin receptors and their signaling in different types of cells should also help clarify the specific effects of EGCG in obesity and thereby enable

Table 1
Effects of (−)-EGCG on receptor in cell-free or cell culture systems[a] (Ref. [22])

Receptors	Activity/expression	Kd or EC_{50}	Models
Adhesion receptor: 67-kDa LR	↓ Binding to laminin	Kd = 39.9 nM	*In vitro*, A549
Cell death receptor: FAS/APO-1 receptor	↑ Expression	50–100 μM	HepG2 cell
Growth factor receptors: EGF receptor	↓ Activity	1.1 μM	Cell-free
FGF receptor	↓ Activity	2.2 μM	Cell-free
HER-2/neu	↓ Activation	22 μM	YCU-H891
IGF-I receptor[b]	↓ Activity and expression	44 μM	SW837
	↓Activity	50 μM	3T3-L1 cell
	↑ Activity	50 μM	Hep G2 cells
IGF-II receptor	↓ Association with G protein	20 μM	3T3-L1 cell
Insulin receptor[a]	↓ Activity	50 μM	3T3-L1 cell
	↑ Activity	50 μM	H4IIE cells
PDGF receptor	↓ Activity	2.3 μM	Cell-free
VEGF receptor	↓ Activation	−13.6 μM	B-CLL cells
Steroid receptors: androgen receptor	↓ Transcription	10–20 μM	LNCaP
Estrogen receptor μ[c]	↓ Binding for E_2	480 μM	*In vitro*
	↑ Gene expression	28 μM	MCF-7
Estrogen receptor β[c]	↓ Binding for E_2	97 μM	*In vitro*
	↑ Gene expression	19 μM	MCF-7 cell
Others: AH receptor	↓ Transcription	<50 μM	Hepa cell
IgE receptor	↓ Expression	−50 μM	KU812 cell
LDL receptor	↑ Expression	<10 μM	HepG2, HeLa
PPARγ	↓ Expression	10 μM	3T3-L1 cell
	↓ Expression	100–400 μM	3T3-L1 cell
TLR-4	↓Activation	<5 μM	AGS cells

[a] Cell lines: A549, human lung cancer cells; HepG2, hepatocellular carcinoma cells; YCU-H891 cells, human nasopharyna carcinoma; SW837 cells, human cobrectal 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 adenocaicinoma cells; AGS cells, human gastric cancer cells. Abbreviations: EGF, epidermal growth factor; AH receptor, aryl hydrocarbon receptor; IgE, immunogbbulin E; LDL, low-density lipoprotein; PPARγ, peroxisome proliferators activated receptor γ; TLR-4, toll-like receptor 4; E2, 17β-estradiol; ↑, increased; ↓, decreased.

[b] Using the immunoprecipitation method, 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 20–180 min. This study on liver cells is different from those of SW827 human cobrectal 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 [3H]-17 estradiol for binding to ERα and ERβ and to elicit receptor gene activity in MCF-7 cells transiently transfected with either chimeric ERα and ERβ.

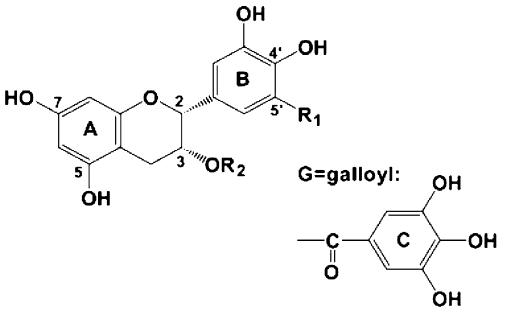
better utilization of one of the oldest medicines in use today.

1.4. The aim of this review

The obesity-preventive effects of green tea and its main compound EGCG are widely supported by results from epidemiological, cell culture, animal, and clinical studies in the recent decade. This paper reviews the evidence for the connections between EGCG and obesity based on various *in vitro* and *in vivo* studies. The possible relevance of each of the proposed mechanisms on human obesity prevention is also discussed in light of current bioavailability data for EGCG and the potential limitations of animal models of obesity. The mechanistic results discussed in this review may possibly be utilized in the treatment of obesity using EGCG.

2. Structure and origin of green tea

Tea is second only to water as one of the most widely consumed beverages in the world, and its origins date back thousands of years. The legendary Chinese emperor, Shen Nung, discovered the detoxifying



	R_1	R_2	g/mol
(-)Epicatechin (EC)	H	H	290
(-)Epigallocatechin (EGC)	OH	H	306
(-)Epicatechin-3-gallate (ECG)	H	G	442
(-)Epigallocatechin-3-gallate (EGCG)	OH	G	458

Fig. 2. Structures of four major green tea catechins. Differences among these catechins occur in the number of hydroxyl groups, the presence of a galloyl group, and the molecular weight (Ref. [30]).

and health-maintaining properties of green tea around 2700 BC [29]. Green tea, which is prepared by drying fresh tea leaves, contains a number of bioactive components, including polyphenols, caffeine, amino acids, and other trace compounds such as lipidsand vitamins [18]. Green tea polyphenols consist mainly of catechins, including EGCG, which is the most abundant and strongest bioactive chemical [8]. GTCs are polyphenolic flavonoids that were once called vitamin P [7]. Since the discovery that they have unique chemical structures (Fig. 2) and are major ingredients of unfermented tea [30], they have been found to possess widespread biological functions and health benefits [31–35].

3. Specific mechanisms of EGCG on adipocytes

3.1. Anti-adipogenic effect of EGCG via extracellular signal-regulated kinase (ERK)- and cyclin-dependent kinase (CDK)-dependent signaling pathway

The MAPK family is an essential part of the signal transduction machinery in signal transmissions from cell surface receptors and environmental stimulation, and it contains three major MAPK subfamilies: ERK, p38, and JNK [36]. They have been proposed to serve as signal elements in several types of cells through which EGCG may regulate cell growth [10,13] and may modulate the mitogenicand adipogenic signalings of IGF-I in 3T3-L1 preadipocytes [37]. Also, it is well known that obesity is the result of both increased adipocyte size (hypertrophy) and increased adipocyte number (hyperplasia) [29]. Therefore, the anti-adipogenic effect of EGCG is important not only in reducing adipocyte differentiation but also in inhibiting adipocyte proliferation, suggesting that two cell cycle control kinases, ERK and cyclin-dependent kinase (CDK), are required for these inhibitory effects [30]. Further supporting this concept was the finding that EGCG induced a decrease in phosphorylated ERK1/2 in 3T3-L1 preadipocytes but did not alter the total levels of MEK1, ERK-1, ERK-2, p38, phospho-p38, JNK, or phospho-JNK (Fig. 3) [30,37], suggesting that EGCG acts on a specific type of MAPK, especially in the ERK MAPK family. This contention is also partially supported by the fact that chronic (24- or 48-h) exposure to EGCG induced a decrease in the phosphorylated ERK1/2 of preadipocytes, although it did not alter total levels of MEK1 or ERK 1/2 proteins [37]. It was generally accepted that resistin (Rstn) is the most recent example of an adipokine with contrasting roles in mouse and man [38]. This protein was initially shown to be released in large amounts from mouse adipocytes; its release was increased in obese mice and accompanied by insulin resistance [39], implying that adipocyte-derived Rstn linked obesity to diabetes. It was generally accepted that Rstn is expressed in white adipose tissue and is induced during adipogenesis [39]. In fact, Steppan et al. reported that Rstn is secreted by mature adipocytes and that resistin has an inhibitory effect on insulin-stimulated glucose uptake in differentiated 3T3-L1 adipocytes [40]. However, Liu et al. reported that intracellular Rstn protein significantly decreased in the presence of 100 μM EGCG 3 h after treatment, whereas the release of the Rstn protein did not significantly change. This suggests that EGCG may modulate the distribution of Rstn protein between the intracellular and extracellular compartments [41]. This result demonstrated that EGCG did not affect the amounts of ERK1/2, phospho-JNK, phospho-p38, and phospho-Akt proteins but reduced the amounts of phospho-ERKl/2 proteins. Moreover, Hung et al. reported that EGCG down-regulated adipocyte differentiation through the CDK2 signaling pathway [30]. These results suggested that the antimitogenic effect of EGCG on 3T3-L1 preadipocytes is dependent on the ERK MAPK and CDK2 pathways and is likely mediated through decreases in their activities. Future studies on discovering the EGCG receptor in fat cells and on characterizing its oxidative stress are also needed to elucidate the mechanisms of how EGCG signals reduce the activities of MEK1 and CDK2 proteins.

3.2. Anti-adipogenic effect of EGCG via activation of AMP-activated protein kinase (AMPK)

AMP-activated protein kinase (AMPK) represents a metabolite-sensing protein kinase that shares amino acid

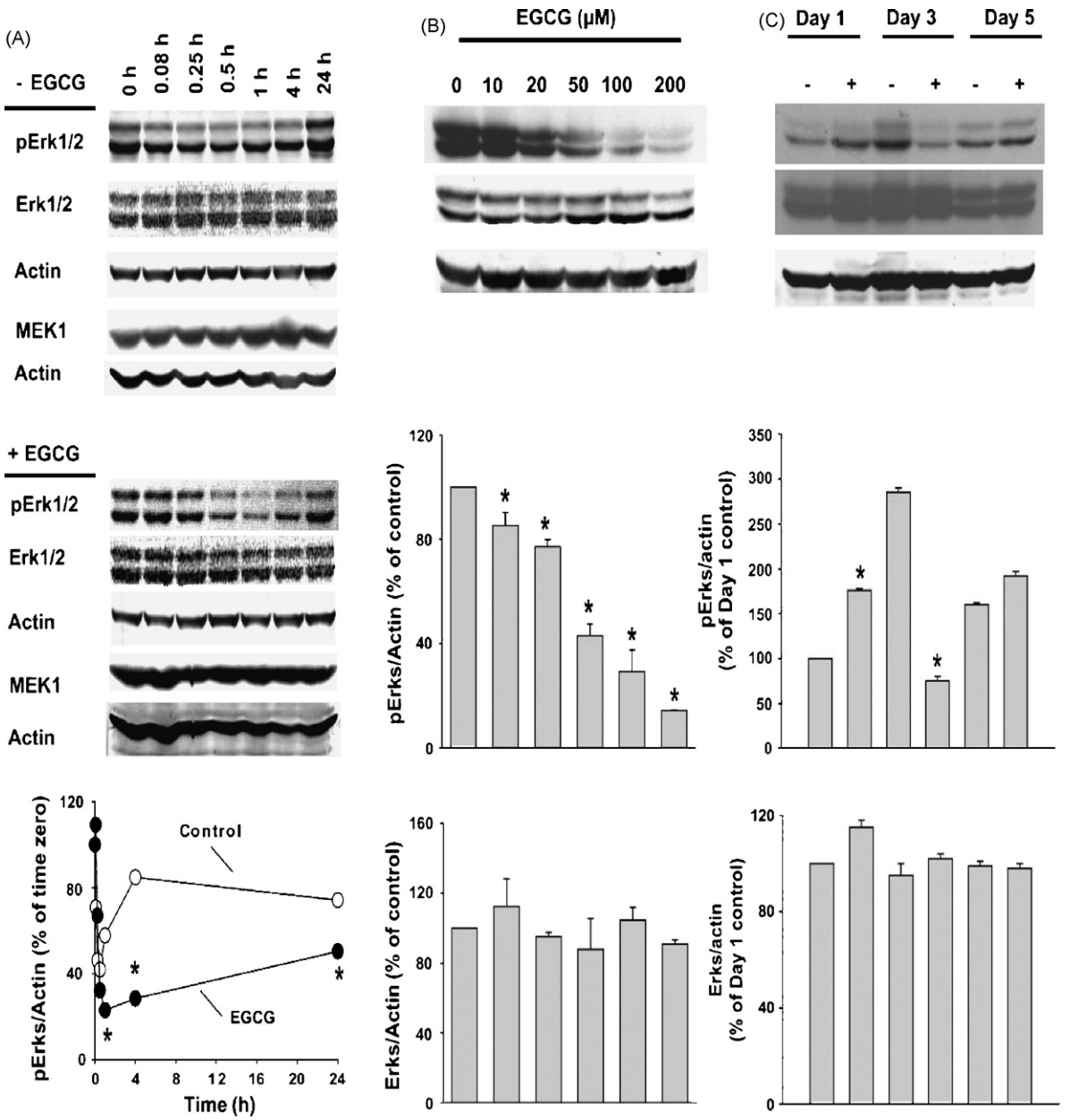


Fig. 3. Effects of EGCG on protein amounts of ERK-1, ERK-2, phospho-ERK-1, phospho-ERK-2, and MEK1 in 3T3-L1 preadipocytes (day 3). (A) Time-dependent effect of EGCG at 50 μM was observed. (B) Dose-dependent effect of EGCG was observed after 4 h of treatment. (C) EGCG at 50 μM altered MEK1 activity, as indicated by decreased phospho-ERKs, which was dependent on the growth phases of cells after 4 h of treatment. Day 1, the day when cells were plated; latent, day 3; log-phase, day 5 (confluent); ERK1/2, ERK-1 + ERK-2; pERKl/2 or pERKs, phospho-ERK-1 + phospho-ERK-2. These kinases were measured by Western blot analysis and then expressed after normalization to actin. Data are expressed as means ± S.E. from triplicate determinations. In some data, S.E. bars are too small to be seen. $^{*}P < 0.05$ vs. the control (Ref. [30]).

sequence homology with yeast SNF1 [42]. AMPK is known to play a major role in energy homeostasis by coordinating a number of adaptive responses in ATP-depleting metabolic states such as ischemia/reperfusion, hypoxia, heat shock, oxidative stress, and exercise [43]. AMPK is sensitively regulated by the allosteric binding of AMP under pathological or physiological conditions of ATP depletion [44–46]. The persistent activation of AMPK has been shown to be connected to p53-dependent cellular senescence, suggesting its role as an intrinsic regulator of the cell cycle in mammalian cells [47]. Moreover, AMPK cascades have emerged as novel

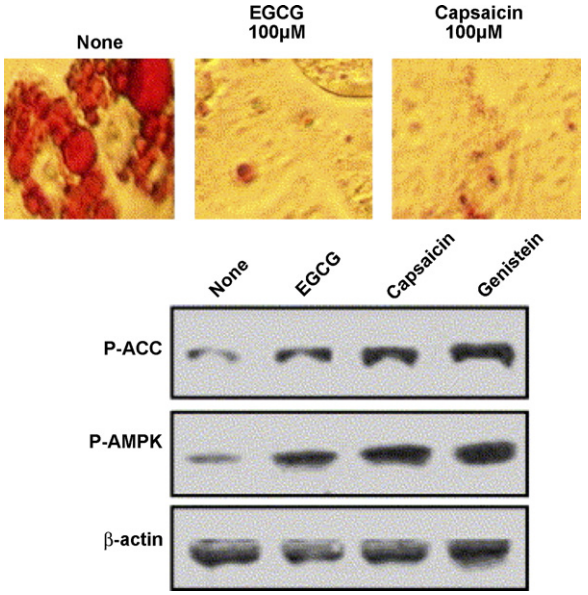


Fig. 4. Similar effects of capsaicin and EGCG on AMPK activation and adipocyte differentiation interference. 3T3-L1 pre-adipocyte were pre-treated with EGCG or capsaicin (100 μM) for 30 min and rapidly incubated with differentiation medium. After each time period, AMPK activation and adipocyte differentiation were detected with either Western blot analysis or Oil-Red O staining (Ref. [54]).

targets for the treatment of obesity and type 2 diabetes [48–50]. AMPK is known to be activated with 5-aminoimidazole-4-carboxamide riboside (AICAR), which is converted to a nucleotide that mimics the effect of AMP, and long-term treatment with AICAR has prevented the development of diabetes in animal models [49]. Also, the pro-apoptotic potential of the activated AMPK was observed in the AMPK over-expressed conditions of various cells [50–53]. Hwang et al. suggested that several naturally occurring compounds have potential anti-obesity effects, showing that either EGCG or capsaicin activated AMPK and inhibited adipocyte differentiation in 3T3-L1 cells (Fig. 4) [54]. This result indicated that AMPK activation is necessary for the inhibition of adipocyte differentiation by EGCG and capsaicin. The mechanism that affected AMPK regulation with physiological stimuli or anti-obesity agents might present a promising target for the development of strategies for the treatment of obesity. Also, AMPK cascades have been postulated to respond to the intracellular level of AMP or to the AMP:ATP ratio and to be highly sensitive to oxidative stress [44]. Generally, reactive oxygen species (ROS) have been suggested as upstream molecules of AMPK-activated signals [43]. In fact, genistein inhibits adipocyte differentiation and the induction of adipocyte apoptosis through the activation of AMPK paralleled with the generation of ROS, and this effect was similar to that of EGCG treatment [54]. As seen in Fig. 5, AMPK phosphorylation increased 2.4-fold in a time-dependent manner, and its substrates, such as acetyl-CoA carboxylase, were also enhanced. Also, AMPK and acetyl-CoA carboxylase were significantly activated by genistein in a concentration-dependent manner. One of the AMPK activation mechanisms was suspected to be ROS, since it was recently reported that various therapeutic effects in naturally occurring compounds involve the release of ROS [53]. In fact, genistein significantly induced ROS generation, which led to AMPK activation, and these effects were abolished by NAC (5 mM) treatment (Fig. 5) [54]. These results indicate that ROS is necessary for AMPK activation in the inhibitory process of adipocyte differentiation by phytochemicals in 3T3-L1 cells. Overall, the anti-proliferatory and lipolytic effects of EGCG have been attributed to their ability to modulate various signaling pathways, specifically those that control cell proliferation and survival.

3.3. Anti-adipogenic effect of green tea via inhibition of lipogenic enzymes

GTCs are found to possess anti-lipogenic activity (Table 2) [22]. 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), glyceol-3-phosphate dehydrogenase (G3PDH), and stearoyl-CoA desaturase-1 (SCD-1) [22,11,10,55]. ACC is the rate-limiting step in fatty acid synthesis for catalyzing the conversion of acetyl-CoA to malonyl-CoA [56]. When green tea ECG or EGCG was incubated with rat liver ACC, both catechins (with Ki of 310 μM) inhibited the activity of ACC [56]. However, (+)-catechin, EC, and EGC, do not have that effect. A recent report also showed that EGCG supplementation down-regulates ACC mRNA expression in obese mice [10]. It has been already reported that 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 IC50 of 52 μM [57]. Generally, EGCG's inhibition of FAS activity is composed of reversible fast-binding inhibition and irreversible slow-binding inactivation [57]. 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 [58]. In addition, EGCG is reported to suppress FAS mRNA and protein levels expressed by MCF-7 breast cancer cells, and EGCG signaling may be involved in the down-regulation of the

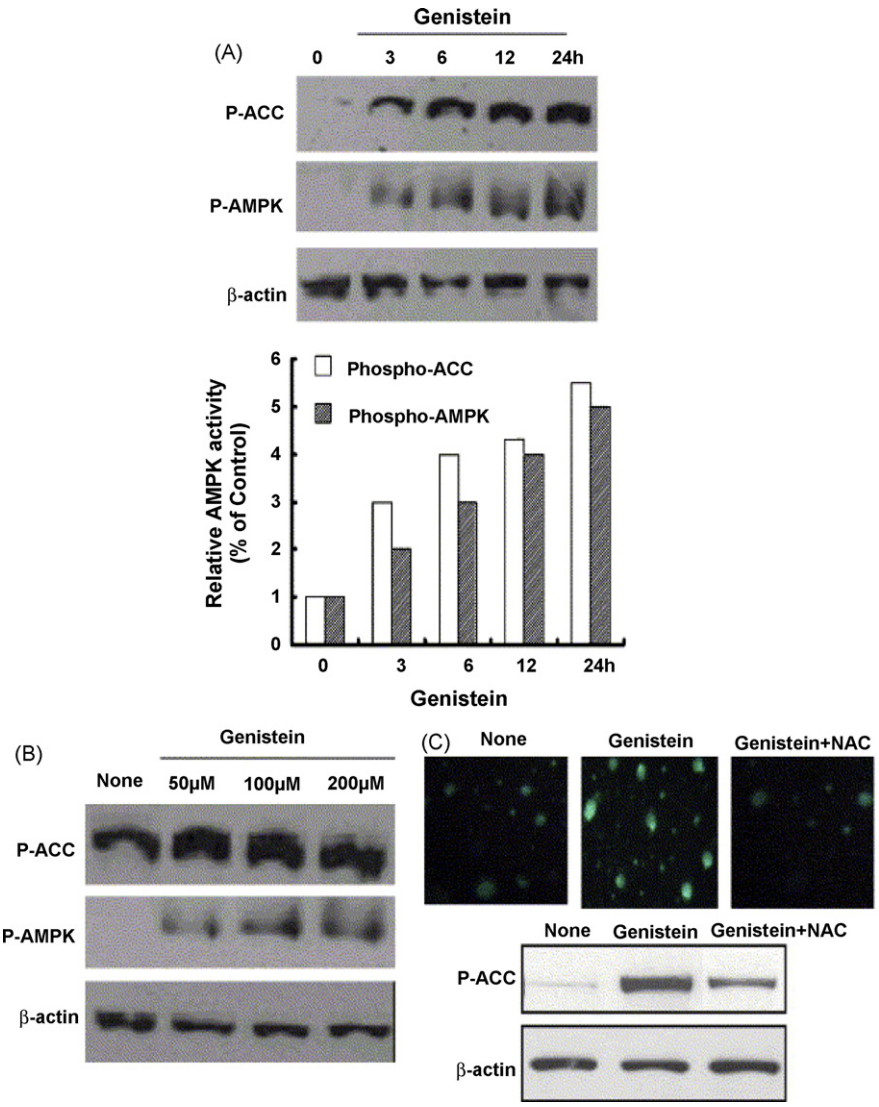


Fig. 5. The effects of AMPK activation by genistein. 3T3-L1 cells were fully differentiated by differentiation cocktail, cells were exposed to genistein (100 μM) for the indicated time periods (A) or various concentrations, respectively, (B) and AMPK activation and its substrate acetyl-CoA carboxylase (ACC) phosphorylation were detected by Western blot analysis. Also differentiated cells were exposed to genistein for 12 h in the presence or absence of NAC (5 mM). After additional incubation for 30 min in the presence of 10 μM DCFH-DA the changes in fluorescence intensity were measured by fluorescence-activated cell scanning analysis. Under the same conditions, the phosphorylation level of ACC-Ser^{79} (P-ACC) were examined (C) (Ref. [11]).

EGF receptor and its downstream Akt and Sp-1 proteins [59]. Moreover, a decrease in the expression of hepatic ME and G6PDH, two enzymes that generate NADPH for fatty acid biogenesis, has also been observed in obese mice treated with EGCG [10]. Furthermore, the activity and expression of G3PDH, the rate-limiting step in TG biosynthesis, are decreased by EGCG treatment [60]. Also, it was found that gene expression of SCD1, the rate-limiting enzyme in the synthesis of monounsaturated fatty acids by liver and adipose tissue, was suppressed in EGCG-treated obese mice [10]. Overall, EGCG or green tea appears to reduce fatty acid and TG synthesis by inhibiting lipogenic enzymes, and this may explain the hypolipidic liver, fat cells, and blood.

3.4. Anti-adipogenic effect of green tea via down-regulation of adipocyte marker proteins and its target genes

Generally, the enforced expression of PPARγ and C/EBPα stimulates adipogenesis in NIH 3T3 fibroblasts, suggesting the essential roles of these transcription

Table 2
EGCG inhibition of lip id-related enzymes in cell-free systems[a] (Ref. [22])

Enzymes	IC_{50} (μM)
Lipogenic enzymes	
ACC	310
Aromatasc	60
FAS	52
Lanosterol 14α-demethylase	>100
Oxidosqualene: lanosteral cyclase	>100
Squalene epoxidase	0.7
Lipolytic enzymes	
GL[b]	10
PL	0.34–11
Oxidoreductase	
Glycyrrhizin-binding lipoxygenase	10
Lipoxygenase	10
Type 1 5α-reductase	15
Type 2 5α-reductase	74
Others	
COMT	0.2

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

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

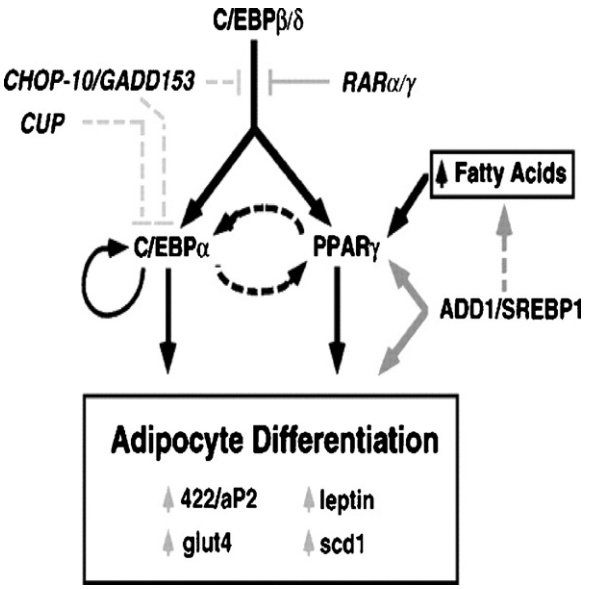


Fig. 6. Interactions among transcription factors in the control of adipocyte differentiation. Positive (↓) and negative (⊥) interactions between transcriptional factors that combine to control differentiation are schematically represented. Those interactions that are less clear are depicted with broken lines (Ref. [63]).

factors in regulating adipogenesis [61,62]. As shown in Fig. 6, PPARγ2 and C/EBPα are found almost exclusively in the adipose tissue and have been linked to the adipocyte differentiation [63]. Also, PPARγ2 and C/EBPα are found almost exclusively in the adipose tissue and have been linked to adipocyte differentiation [62], indicating that these factors play a crucial role both in the induction of adipose-specific genes and in the manifestation of the mature adipose phenotype. Moreover, the γ subtype of PPAR is expressed at a high level in the adipose tissues of mice, and its expression rapidly and dramatically increased during the differentiation of 3T3-L1 preadipocytes [64]. Furthermore, the combined expression of PPARγ and C/EBPα have synergistic effects in promoting fat cell conversion in myoblasts [64,65], indicating that these genes are very important for adipocyte fat accumulation. These transcription factors coordinate the expression of genes involved in creating and maintaining the adipocyte phenotype, including aP2 [61]. The aP2 is a member of the intracellular lipid binding protein family, suggesting that this protein is involved in the formation of atherosclerosis predominantly through the direct modification of macrophage cholesterol trafficking and inflammatory responses [61,62]. Tontonoz et al. reported that several genes specialized for lipid metabolism and storage, many of which contain functional PPAR-response elements, are induced in the adipogenic differentiation process, and found that aP2, which is a target gene of terminal adipocyte differentiation involved in FFA transportation and shunting within the cell, is one such gene [66]. In fact, Hammarstedt et al. demonstrated that fat cells from insulin-resistant individuals display decreased expression of PPAR target genes, such as aP2, and increased lipid partitioning, with a subsequent enlargement of the existing adipose cells [67]. By contrast, Kao et al. reported that EGCG reduced body weight and body fat [17]. As shown in Figs. 7 and 8, the control rats continued growth and increased their body weight by 25–34% relative to their initial weight, whereas female Sprague-Dawley rats injected daily ip with 12.5 mg EGCG (∼92 mg/kg BW) lost 10% of their body weight relative to their initial weight and 29% relative to the control weight after 7 days of treatment. Also, it was demonstrated that EGC and EGCG significantly down-regulated the expression of adipocyte maker genes during adipocyte differentiation [13], indicating that the negative effect of EGCG on adipogenesis was accompanied by the reduction of PPARγ2 protein in 3T3-L1 cells, which was accompanied by the attenuation of C/EBPα expression. Because PPARγ is a key transcription factor in the induction of adipogenesis and lipid accumula-

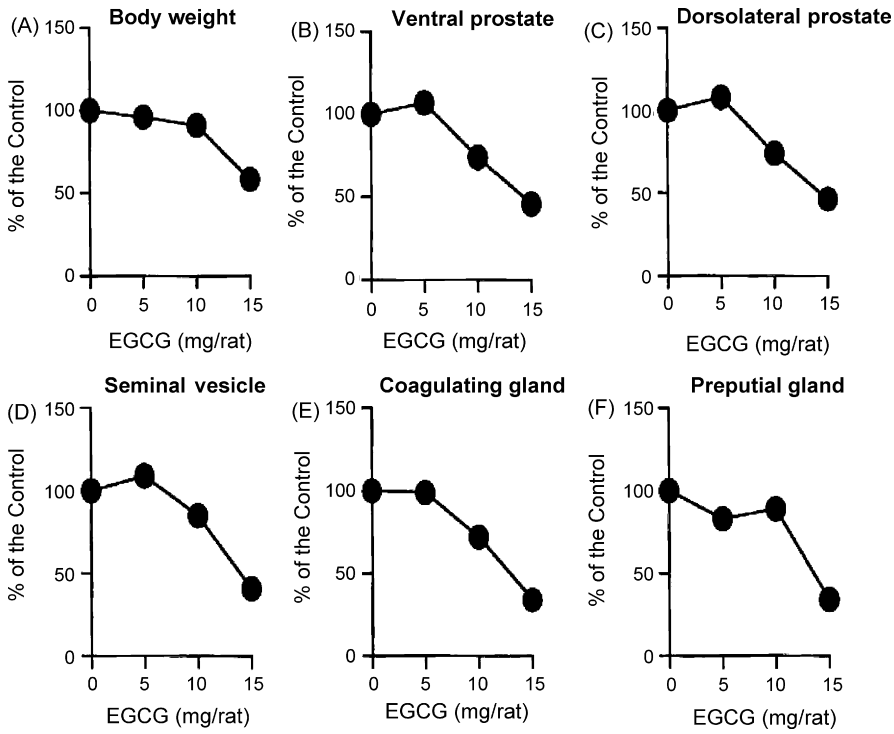


Fig. 7. Dose-dependent effects of EGCG on body weight (A) and weights of the ventral prostate (B), dorsolateral prostate (C), seminal vesicle (D), coagulating gland (E), and preputial gland (F) of male Sprague–Dawley rats that were injected ip with the indicated doses of EGCG daily for 7 days. The 5-, 10-, and 15-mg doses of EGCG injected per rat correspond to about 26, 53, and 85 mg/kg BW, respectively. Data are a percentage of the control value calculated from mean values from five animals by comparing body and organ weights of treated rats to those of control rats after 7 days of treatment. The average ending body and organ weights of control rats were: body weight, 243 ± 4 g; ventral prostate, 133 ± 10 mg; dorsolateral prostate, 104 ± 6 mg; seminal vesicle, 171 ± 14 mg; coagulating gland, 51 ± 4 mg; preputial gland, 119 ± 11 mg. If comparisons are made to starting weights instead of to weights on day 7, the decrease seen with 15 mg EGCG will be smaller. The average starting body and organ weights of control rats were: body weight, 185 ± 4 g; ventral prostate, 123 ± 6 mg; dorsolateral prostate, 91 ± 8 mg; seminal vesicle, 120 ± 12 mg; coagulating gland, 44 ± 2 mg; preputial gland, 100 ± 15 mg (Ref. [17]).

tion [64,65], EGCG-induced down-regulation of PPARγ expression is likely to function similarly to the observed negative effects of EGCG on lipid accumulation and on adipocyte differentiation [14–17]. TG hydrolysis proportionally released glycerol and FFA from adipocytes, and the glycerol release caused the lipolysis in the adipocytes [68]. Generally, natural compounds such as conjugated linoleic acid (CLA) and forskolin induced lipolysis in adipocyte models [29]. In fact, we previously reported that CLA had an anti-adipogenic effect and induced lipolysis in 3T3-L1 cells [65]. However, Wolfram et al. reported that GTCs strongly reduced adipocyte differentiation but did not induce lipolysis [69], indicating that the anti-adipogenic effects of EGCG are not mediated via increased lipolysis.

3.5. Insulin-potentiating activity by green tea

In addition to the anti-adipogenic effects of EGCG on adipocyte marker protein expression, Wu et al. reported that, after 12 weeks of green tea supplementation, the fasting plasma glucose and insulin levels in the green tea group were significantly lower than those in the control group [70]. As shown in Fig. 9, during the 2 h following glucose ingestion, no difference was seen in the plasma glucose levels between the two groups; however, the plasma insulin levels of the green tea group at all time points were significantly lower than those in the control group. In fact, the AUCs for plasma glucose and insulin were 346 ± 13 mg h dL^{-1} and 60 ± 18 μU h mL^{-1} in the control group, respectively, and 336 ± 11 mg h dL^{-1} and 38 ± 10 μU h mL^{-1} in the green tea group, respectively. No significant difference was found between the two groups in the AUC for plasma glucose ($P = 0.260$), but the AUC for insulin in the green tea group was significantly lower ($P = 0.004$) than that in the control group, showing that the green tea group had increased insulin sensitivity. These results indicated that green tea supplementation in the form of regular tea infusions could increase insulin sensitivity in rats by increasing the glu-

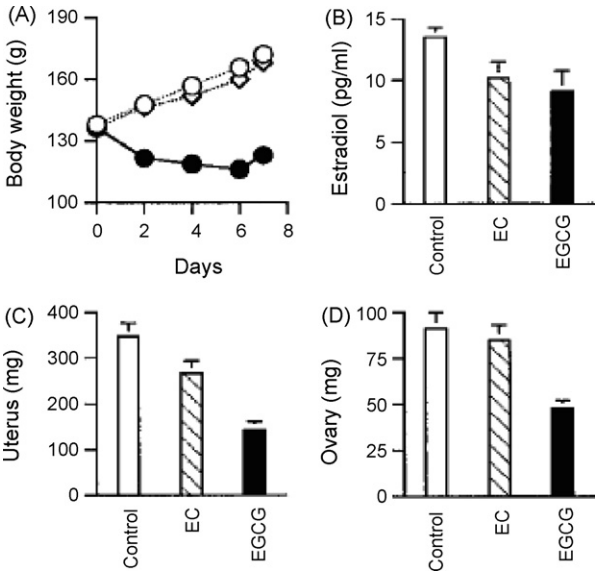


Fig. 8. Differential effects of EC and EGCG on body weight (A), serum 17β-estradiol (B), and weights of the uterus (C), and ovary (D) in female Sprague–Dawley rats. Rats were injected ip with the indicated catechin, 12.5 mg/rat (92 mg/kg BW), daily for 7 days. Values are the mean ± S.E.M. from five animals in each group. The S.E. bar is either too small to be seen or, for clarity, is not shown. Symbols in (A) correspond to control (◯), EC (◊), and EGCG (●) groups (Ref. [17]).

cose uptake and insulin binding of adipocytes. Generally, the most widely known health benefits of tea relate to the polyphenols as the principal active ingredients in protection against oxidative damage and in antibacterial, antiviral, anticarcinogenic, and antimutagenic activities, but polyphenols in tea may also increase insulin activity [70]. Several known compounds found in tea were shown to enhance insulin, with the greatest activity due to EGCG, followed by ECG, tannins, and theaflavins [70–72]. As support for this result, Anderson et al. reported that the majority of the insulin-potentiating activity for green and oolong teas was due to EGCG, showing that the addition of 5 g of 2% milk per cup decreased the insulin-potentiating activity by one-third and the addition of 50 g of milk per cup decreased the insulin-potentiating activity ∼90% [71]. These data demonstrated that tea contains *in vitro* insulin-enhancing activity and that the predominant active ingredient is EGCG. Insulin secretion by pancreatic beta-cells is stimulated by glucose, amino acids, and other metabolic fuels [72]. Li et al. reported that EGCG does not affect glucose-stimulated insulin secretion under high energy conditions where glutamate dehydrogenase (GDH) is probably fully inhibited, and further showed that these compounds act in an allosteric manner independent of their antioxidant activity and that the beta-cell stimulatory effects are directly correlated with glutamine oxidation [73]. These results indicated that EGCG facilitated the dissecting of the complex regulation of insulin

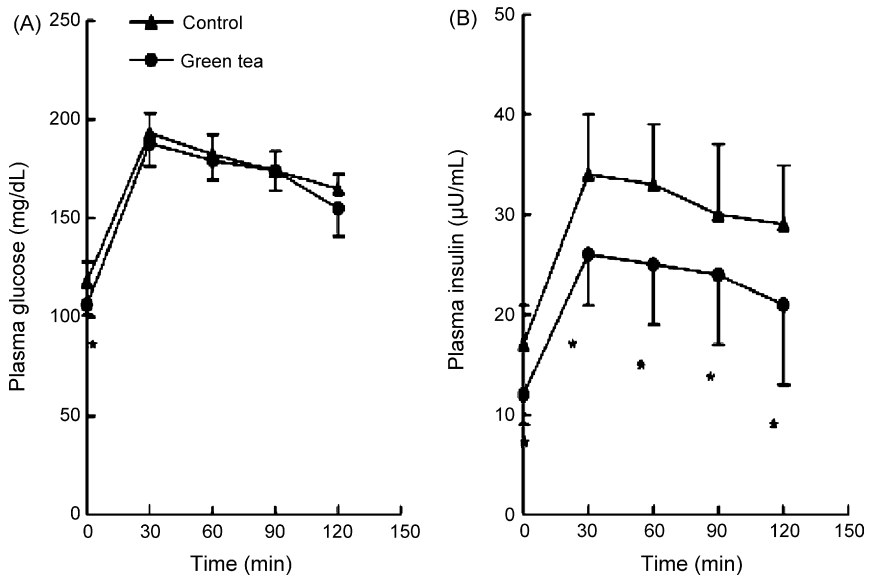


Fig. 9. Changes in plasma levels of glucose (A) and insulin (B) in rats in response to an oral glucose tolerance test (2 g of glucose/kg of BW) performed after 12 weeks with or without green tea supplementation. Values are shown as the mean ± S.D. $^{*}P < 0.05$ compared to the control group (Ref. [70]).

secretion by pharmacologically modulating the effects of GDH.

4. Epidemiological observation of green tea and its clinical study

Although some epidemiological studies have not provided clear-cut evidence for a link between tea consumption and body weight [74], several studies have shown that tea intake is associated with decreased serum concentrations of total cholesterol and lipoprotein cholesterol level. For example, Tokunaga et al. reported that green tea consumption was inversely associated with serum levels of total cholesterol and LDL cholesterol, but not with body weight index, HDL cholesterol, and TG [75]. In another study with men over 40 years of age, higher levels of green tea consumption were associated with an increased proportion of HDL cholesterol together with a decreased proportion of low and very low LDL cholesterol and serum concentrations of total cholesterol and TG, but not with the body weight index [76]. In a recent clinical study, 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 [77]. Also, Nagao et al., reported that the subjects who ingested one bottle of oolong tea containing 690 mg GTCs per day for 12 weeks had a lower body weight, body weight index, waist circumference, body fat mass, and subcutaneous fat area than did the subjects who ingested one bottle of oolong tea containing 22 mg catechins per day [78]. These early studies indicated the possible beneficial effects of GTCs to reduce human body weight. Also, Nakagawa et al. clearly demonstrated that 18 healthy Japanese men were given a green tea extract containing 254 mg and, 1 h after administration, their plasma level of EGCG reached 0.27 nM, while plasma phospholipids, total cholesterol, and RG did not change. However, the plasma phatidycholine hydroperoxide level decreased from 74 pM in controls to 45 pM in EGCG-treated subjects, suggesting that tea catechins are as effective as anti-oxidants [79]. By contrast, there are controversial observations of green tea's regulation of energy expenditure and fat oxidation in humans. In fact, in a respiratory chamber study, ten healthy men had a green tea extract that contains 50 mg caffeine and 90 mg EGCG at breakfast, lunch, and dinner [18]. The results showed that 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. However, in another 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 contained caffeine (104 mg/day) and EGCG (323 mg/day) and were given for 13 week was not associated with weight maintenance after a 7.5% body weight loss in very-low-energy-diet subjects [80]. This study also showed that habitual caffeine consumption affected weight maintenance in the green tea treatment. In addition, these clinical observations indicated that long-term, but not short-term, oral consumption of green tea appeared 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, and the physiological condition of the subjects.

5. Effects of EGCG on the liver and its mechanism

It was generally accepted that the synthesis of fatty acid is the key step for lipogenesis, which is responsible for the complete synthesis of palmitate from acetyl CoA in the cytosol [81]. In rats, the pathway is well represented in adipose tissue and liver. In most mammals, glucose is the primary substrate for lipogenesis; inhibition of lipogenesis occurs in type 1 diabetes mellitus, and variations in its activity may affect the nature and extent of obesity [82]. The suppression of lipogenesis by oolong, black, pu-erh, and green tea leaves in rats has been demonstrated by the suppression of plasma TG, cholesterol, and LDL-cholesterol in experimental animals [83], indicating that pu-erh tea and oolong tea could lower the levels of TG more significantly than could green tea and black tea; meanwhile, pu-erh tea and green tea were more efficient than oolong tea and black tea in lowering the level of total cholesterol. In addition, FAS plays a central role in *de novo* lipogenesis in mammals by the action of its seven active sites, and FAS catalyzes all the reactions in the conversion of acetyl CoA and malonyl CoA to palmitate [81]. FAS concentration is also sensitive to nutritional and hormonal status in lipogenic tissues such as the liver [84]. The *in vivo* suppression of FAS by tea and tea polyphenols has been demonstrated in HepG2 hepatocellular carcinoma cells [85]. The results showed that FAS is over-expressed in the malignant HepG2 hepatocellular carcinoma cells, and its expression is further enhanced by epidermal growth factor (EGF), whereas the expression of FAS was suppressed by EGCG and the expression of FAS was

significantly suppressed by TF-1, TF-2a, TF-2b, and TF-3 at both for cell lipogenesis and proliferations. Additional experimental results demonstrated that EGF-induced biosynthesis of lipids, including TG, cholesterol and fatty acids, and proliferation were significantly suppressed by EGCG and TF-3. In addition, one of the intracellular signaling pathways that is frequently activated in cancer cells is the PI3K/Akt kinase pathway [86]. This pathway has been documented as an important signaling mechanism for cell survival, cell transformation, and tumor growth. In general, Akt is phosphorylated and activated by phosphatidylinositol-3,4,5-triphosphate-dependent kinase (PDK), ultimately resulting in the stimulation of cell growth and survival through transcription activation of the FAS gene [86]. Yeh et al. reported that the activated Akt may bind on the Sp-1 site that leads to transcription of the FAS genes, whereas suppression of FAS expression was due to tea polyphenols including EGCG, TF-1, TF-2, and TF-3 [63]. These findings suggest that tea polyphenols suppress FAS expression by down-regulating the EGF receptor/PI3K/Akt/Sp-l signal transduction pathway.

6. Summary and future directions

Obesity is associated with high blood cholesterol and a high risk for developing diabetes and cardiovascular disease. Therefore, the management of body weight and obesity are increasingly considered important to maintain healthy cholesterol profiles and to reduce cardiovascular risk. Several drugs are used for the therapy of obese-related metabolic diseases, and frequently the possibility of preventing body fat accumulation with these drugs is discussed. Increasing interest in the health benefits of tea has led to the inclusion of tea extracts in dietary supplements and functional foods among these substances of interest. Specifically, EGCG was known to have a beneficial effect on human health that reduced adipocyte differentiation and decreased TG levels. However, epidemiologic evidence regarding the effects of tea consumption on obesity-related disease are conflicting. This is an important area for future investigations, as is the elucidation of EGCG signaling events during adipogenesis. Although more studies are required to examine the effects and mechanisms of EGCG in animals and humans, the anti-adipogenic effect of green tea, especially EGCG on adipocyte differentiation may provide an effective treatment for obesity. Also, the mechanistic results discussed in this review may possibly be utilized in the treatment of obesity using EGCG.

Acknowledgments

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