

# SYSTEMATIC REVIEW: HYPOLIPIDEMIC ACTIVITY OF OOLONG TEA POLYMERIZED POLYPHENOLS

Sudathip Sae-tan

Department of Food Science and Technology, Faculty of Agro-Industry, Kasetsart University, Bangkok, 10900, Thailand

## ABSTRACT:

Oolong tea (*Camellia sinensis*, Theaceace) is a partially-fermented tea mostly consumed in Asia. It has been known for high antioxidant content and its medicinal use since ancient times. Oolong tea has been studied for its beneficial health effects including the reduction of oxidative stress, anti-cancer, anti-diabetes, prevention of atherosclerosis, heart disease, hypertension, anti-obesity and modulation of dyslipidemia. Dyslipidemia is a condition characterized by changes in blood lipid profile. It consists of increased triglycerides (TG) and free fatty acid (FFA), decreased high-density lipoprotein (HDL) associated cholesterol with HDL dysfunction and normal or slightly increased low-density lipoprotein (LDL) associated cholesterol with increased small dense LDL and increased plasma apolipoprotein (apo) B. Oolong tea uniquely contains oolong tea polymerized polyphenols (OTPPs). Many human studies have focused on OTPPs in oolong tea as an active agent in modulation of dyslipidemia. Overall, they have shown that OTPPs-enriched oolong tea could modulate dyslipidemia by lowering serum triglycerides, total cholesterol and LDL cholesterol. These effects could contribute to lowering the risk of cardiovascular disease and renal disease. Several mechanisms have been proposed based on these studies and include: inhibition of pancreatic lipase, reduction of lymphatic triglyceride absorption in small intestine and increase of energy expenditure. In the present review, the current state of science with regard to clinical studies of oolong tea and OTPPs and dyslipidemia markers is discussed. I attempt to critically evaluate the available information and point out the future research area. Although considerable information is available, questions remain in terms of the toxicity and primary mechanism of action and the dose-response relationships involved. The information reviewed here suggests that, even though OTPP-enriched oolong tea could benefit to dyslipidemia, maintaining varied healthy diets and active physical activities is the best approach to have the healthy life.

**Keywords:** Dyslipidemia, Hypolipidemic activity, Obesity, Oolong tea, Oolong tea polymerized polyphenols, Polyphenols, Tea

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## INTRODUCTION

The problem of obesity has rapidly increased and become a major worldwide health issue. One of the strong risk factors for the development of obesity is consumption of high-fat diet. Epidemiological studies have shown that obesity is generally more prevalent in societies that consume a Western-style diet, in which higher intakes of red meats, high-fat dairy products, and refined grains [1, 2].

Overweight and obesity is a condition of abnormal

or excessive fat accumulation around abdominal cavity, defined as a Body Mass Index (BMI) [3]. Obesity not only promotes mortality and morbidity but also is associated with increased medical and economical costs [4, 5]. Overweight and obesity are major risk factors for a number of chronic diseases, including type 2 diabetes, coronary artery disease, hypertension, respiratory disorders and dyslipidemia [5, 6]. A typical dyslipidemia of obesity consists of increased triglycerides (TG) and free fatty acid (FFA), decreased high-density lipoprotein (HDL) associated cholesterol with HDL dysfunction and normal or slightly increase low-density lipoprotein (LDL)

\* Correspondence to: Sudathip Sae-tan  
E-mail: fagists@ku.ac.th

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associated cholesterol with increased small dense LDL and increased plasma apolipoprotein (apo) B [5, 7]. Age, genetics, smoking, physical activity, stress and diet are risk factors of dyslipidemia. Among these factors, diet is the target of prevention and improvement of dyslipidemia because it is modifiable.

Tea (*Camellia sinensis*, Theaceae) is the second most popular beverage in the world, next to water. Tea has been used for medical purposes since ancient times. Globally tea production has increased significantly by 6 percent to 5.07 million tons in 2013 and globally tea consumption has increased by nearly 5 percent in 2013 to 4.84 million tons [8]. Based on different degree of fermentation, there are three main types of tea, including black, oolong and green tea. They differ in terms of processing and chemical compositions. Black tea is the main product of world tea production. It is fully fermented, made by crushing tea leaves to release the polyphenol oxidase and peroxidase for fully catalyzing the enzymatic oxidation and polymerization of original tea catechins. Oolong tea is partially-fermented. It is made by wilting fresh leaves by sun, cooling, tossing, withering, fixation, rolling and drying [9]. Green tea is unfermented tea produced from fresh tea leaves and enzymatic oxidation is inhibited by steaming or pan-frying. They are differently processed in the terms of fermentation which actually refers to oxidative and enzymatic changes within tea leaves. The degree of oxidation in fermentation process affects the polyphenolic profile of the tea. The differences between green, oolong and black tea, regardless the degree of fermentation, is also contributed by their contents of free amino acids, mainly *L*-theanine and several natural amino acids including glutamic acid, asparagine, serine, alanine, leucine, and isoleucine [10, 11].

Dietary, pharmacological and surgical strategies have been developed in the last decades to prevent and treat the metabolic effects of obesity including dyslipidemia, for example, controlling of food intake, increase of energy expenditure, promotion of fat oxidation or inhibition of fat absorption [2]. Although there are many drugs and surgical approaches to treat and prevent obesity-related metabolic effects, they have side effects [12]. Therefore dietary approach seems to be the safest and most cost-effective option for those who are obese.

Tea has been reported for its potential health

benefits against a number of chronic diseases, including cardiovascular disease, cancer, metabolic syndrome, diabetes, and obesity [13]. Many beneficial effects of tea are attributes to the polyphenols. Green tea has only monomeric catechins, i.e. (-)-epicatechin (EC), (-)-epicatechin-3-gallate (ECG), (-)-epigallocatechin (EGC), and (-)-epigallocatechin-3-gallate (EGCG). These polyphenols have been proposed to be key players to exert the health benefits of green tea. Black tea and oolong tea contain a mixture of catechins and their oxidized polymeric substances such as theaflavins and thearubigins [10]. Oolong tea has been tested for its various pharmacological features such as antioxidant activity by reducing oxidative stress, anti-cancer, anti-diabetes, preventive effects of atherosclerosis, heart disease, hypertension, anti-allergic effect, anti-septic effects, and anti-obesity [10, 14, 15]. However, the health beneficial effects of oolong tea have not been much reviewed especially regarding its benefits on lipid profile.

In the review, author focus on the potential benefits on lipid profile of oolong tea as an agent for prevention of dyslipidemia. The reviewed studies were collected from MEDLINE, EMBASE, CINAHL, Plus, AMED (The Allied and Complementary Medicine Database), JSTAGE as well as local and Thai database. Keywords used were oolong tea, oolong tea polymerized polyphenols, oolong tea extract, dyslipidemia, obesity. In addition, bibliographies of relevant articles were investigated to identify potential relevant studies. The first study was published in 1992 and the latest was from 2015. This review focuses on human studies and discusses the outcomes of the relevant studies to elaborate the proposed possible mechanisms of oolong tea and oolong tea polymerized polyphenols (OTPPs) to modulate lipid profile.

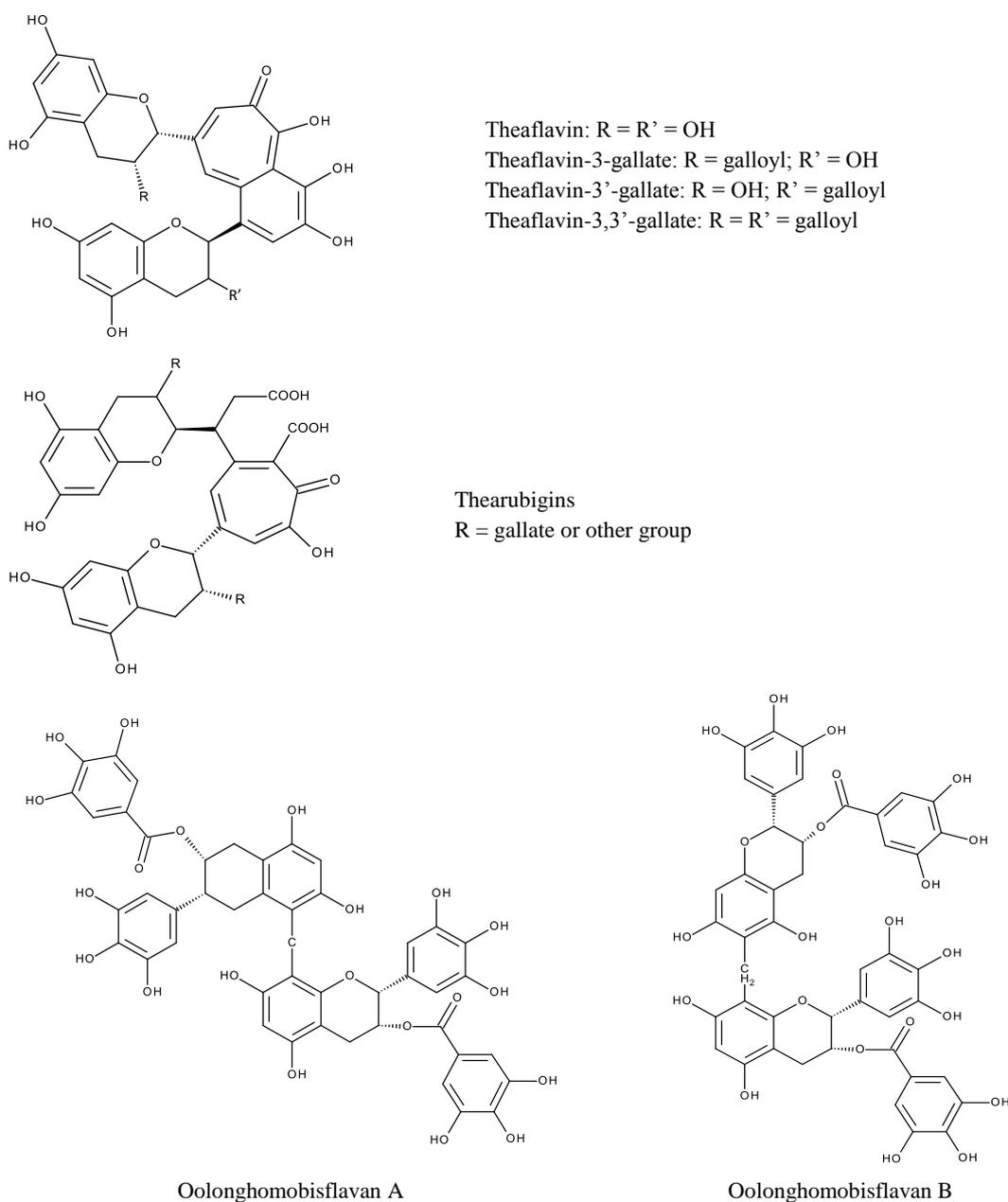
### Dyslipidemia

Dyslipidemia is a disorder characterized by changes in lipid profile in terms of levels and compositions. According to Adult Treatment Panel III, dyslipidemia is observed when a person has plasma total cholesterol  $\geq 200$  mg/dL, LDL cholesterol  $\geq 130$  mg/dL, HDL cholesterol  $< 40$  mg/dL and plasma triglycerides  $\geq 150$  mg/dL [16]. Dyslipidemia results from inborn defects of lipoprotein production or metabolism. However in the most cases, it results from unhealthy lifestyle such as less physical activity, excessive smoking or

**Table 1** Oolong tea compositions

Components	Oolong tea (mg/g)
<b>Caffeine</b>	64
<b>Flavan-3-ol without galloyl moiety</b>	
Galocatechin (GC)	30
Epigallocatechin (EGC)	6
Catechin (C)	10
Epicatechin (EC)	2
<b>Flavan-3-ol with galloyl moiety</b>	
Epigallocatechin gallate (EGCG)	14
Galocatechin gallate (GCG)	16
Epicatechin gallate (ECG)	3
Catechin gallate (CG)	7
<b>Oolong tea polymerized polyphenols (OTPPs)</b>	114

Modified from Toyoda-Ono, et al. [21]

**Figure 1** Structures of oolong tea polymerized polyphenol (OTPPs) in oolong tea

alcohol consumption, diabetes, infection, obstructive liver disorder or medication [17].

Chronic hyperlipidemia is a major risk factor for cardiovascular disease [17, 18]. The pathology is initiated by disruption of the arterial endothelium by high level circulating LDL cholesterol, which leads to overexpression of adhesion and chemoattractant molecules to injured sites, and the recruitment of circulating monocytes to these sites. Oxidized LDL cholesterol stimulates endothelial adhesiveness and promotes T cell and monocyte activation. Oxidized LDL cholesterol is also taken up by macrophages in the artery, which develop into foam cells. Foam cells are characterized as atheromatous plaques [17, 19]. These all processes lead to the development of cardiovascular disease.

### Chemical components of oolong tea

The main components in oolong tea are polyphenols, alkaloids, saponins, polysaccharides and *L*-theanines. Caffeine is present at levels of up to 5-6% in oolong tea [20]. Polyphenols found in oolong tea are catechins and its derivatives, including EC, ECG, EGC, catechin (C), gallicocatechin (GC), and EGCG as shown in Table 1.

Oolong tea polymerized polyphenols (OTPPs) are derived from tea catechins by oxidation and polymerization reaction during the semi-fermentation and heating process of production, catalyzed by polyphenol oxidase. The degree of fermentation of polyphenols in oolong tea ranges from 8 to 85% depending on the variety and production style of the tea. OTPPs could not be found in green tea. Therefore it is a unique compound in oolong tea. The representative and the most abundant of OTPPs in oolong tea are thearubigins, theaflavins, dimeric catechins such as oolonghomobisflavan A and oolonghomobisflavan B as shown in Figure 1 [20].

A group of polymeric oxidized flavan-3-ols was isolated and identified from oolong tea as theasinensins A, B, C, D, E, E and G. These compounds formerly known as bisflavanols which were formed by coupling of EGCG and they are proposed to be the bioactive flavonoids in oolong tea. Once tea leaves are crushed and kneaded, theaflavins are formed in the leaves but not theasinensins. The formation of theasinensins is observed in heating process of the leaves at 80°C [10].

### Effects of OTPPs on lipid profile

A recent population-based case-control study

showed that oolong tea consumption could lower the risk of dyslipidemia [22]. The result showed that tea consumption at least 600 mL a day (green, oolong or black tea) was associated with the lowest odds of dyslipidemia risk when compared with non-consumption. Only oolong tea consumption was found to be associated with the low HDL cholesterol levels. This study showed that the risk of dyslipidemia was associated with duration of tea consumption and amount of dried tea leaves brewed in dose-dependent response. The consumption of oolong tea for a longest duration was found to be associated with lower blood total cholesterol, triglycerides and LDL cholesterol. Another recent double-blinded placebo-controlled crossover study in Thailand showed that drinking 500 mL of oolong tea containing OTPPs 70 mg with corn soup containing 40 g of fat reduced postprandial triglycerides compared to placebo group as shown in the incremental blood triglyceride area under the curve [23]. From these two recent studies, it indicates that oolong tea containing OTPPs has beneficial effects on lipid modulation. A number of studies have shown the beneficial effects of OTPPs on lipid profile and they support and agree to each other. The oldest study in human by Chen, et al. [24] reported that oolong tea consumption effectively lowered plasma triglycerides and cholesterol in humans. This report drew attention of many researchers on anti-obesity and hypolipidemic effects of oolong tea. A study by Chen, et al. [25] showed that 15% of obese subjects consuming oolong tea 4 times a day for 6 weeks (2 g/bag in 200-300 mL) reduced body weight more than 3 kg and 67% of subjects had lowered body weight more than 1 kg. Additionally serum triglyceride levels of subjects with high serum triglyceride were lowered by 20% with oolong tea consumption. From these two early studies and the study by Nakai, et al. [26] which they showed OTPPs, including oolonghomobisflavans A and B and oolongtheanine 3'-*O*-gallate, had the strong inhibitory effects against pancreatic lipase and these activities were much higher than EGCG in green tea, scientists paid more attention on oolong tea and its health beneficial effects.

The effects of OTPPs-enriched oolong tea on postprandial serum triglyceride elevation were studied in 22 subjects with serum triglycerides between 100-250 mg/dL in a double blinded cross-over trial [27]. In this study, subjects consumed a high fat food (40 g fat) in the morning with empty

stomach followed by drinking oolong tea enriched with OTPPs (68 mg/245 mL). The results showed that OTPPs-enriched oolong tea consumption significantly reduced elevation of serum triglycerides and serum chylomicron (18.3% and 29.9% as area under the curve, respectively). Although serum remnant-like particle cholesterol and LDL cholesterol were no significantly different between OTPPs-enriched oolong tea treatment and the control drink, area under the curve of those markers tended to decrease. Another study was performed in 12 healthy subjects [28]. The subjects were given potato chips as a high fat source (38 g fat) and drank oolong tea added with OTPPs (69 mg/250 mL) three times a day for 10 days. The results showed that there were no significant effects of OTPPs-enriched oolong tea consumption on serum triglycerides, total cholesterol, HDL cholesterol and LDL cholesterol compared to placebo group. But OTPPs-enriched oolong tea tended to reduce serum triglyceride concentrations.

A study in 96 overweight subjects showed that OTPPs-enriched oolong tea consumption (OTPPs 70 mg/350 mL, three times a day) for 12 weeks significantly reduced LDL cholesterol (6.1%) but there was no significant effects on serum triglycerides, total cholesterol and HDL cholesterol. However, the reduction of serum triglycerides significantly reduced (34.2%) in subgroup with initial serum triglycerides more than 150 mg/dL compared to the effects of placebo group (OTPPs 6 mg/350 mL) [29]. This study also showed OTPPs-enriched oolong tea consumption resulted in the significant reduction of visceral fat area (-9.2 cm<sup>2</sup>) compared to baseline since 8-week consumption. The researchers also recalculated subgroup of subjects and found that the significant reduction of visceral fat was found since 4-week consumption in subjects who had visceral fat area more than 100 cm<sup>2</sup> (categorized as subjects with risk of metabolic syndrome). This suggested that the lowering triglyceride and visceral fat of OTPPs-enriched oolong tea had more effect on subjects with overweight and high level of initial triglyceride concentration. Next year, the same group of researchers also conducted the study to investigate the effects of OTPPs-enriched oolong tea consumption in 258 overweight volunteers [30]. The subjects had OTPPs-enriched oolong tea 1 bottle a day for 12 weeks (OTPPs 70 mg/bottle). The results confirmed with the last study that OTPPs-enriched oolong tea consumption reduced total fat area (-9.4

cm<sup>2</sup>) and visceral fat area (-7.8 cm<sup>2</sup>) compared to baseline since 8-week consumption and the effects was found earlier in subgroup that had initial visceral fat area more than 100 cm<sup>2</sup>. The consumption of OTPPs-enriched oolong tea also significantly reduced serum triglyceride (8.5%), total cholesterol (2.5%), and LDL cholesterol (3.5%) compared to baseline but not HDL cholesterol. A few years later, the effects of OTPPs-enriched oolong tea was conducted again with 300 overweight subjects ( $25 \leq$  body mass index  $\leq 30$  kg/m<sup>2</sup>) [31]. In this study subjects were given 2 serving a day of OTPPs-enriched oolong tea for 16 weeks (OTPPs 70 mg/350 mL/serving). The results showed that the change of total fat area and visceral fat area significantly decreased (-11.32 and -7.01 cm<sup>2</sup>, respectively) compared to baseline since 8-week of OTPPs-enriched oolong tea consumption and the changes were much stronger in subgroup of subjects with visceral fat area more than 100 cm<sup>2</sup>. Only HDL cholesterol significantly reduced in men received OTPPs-enriched oolong tea (3.6%), but not serum triglycerides level, LDL cholesterol and total cholesterol. The results from these three studies which showed the effect of OTPPs-enriched oolong tea at different frequency, indicates that the drinking oolong tea containing 70 mg OTPPs at least 350 mL per day could modulate lipid profile and decrease visceral fat area in overweight subjects and the results were more effective in subgroup with high risk of metabolic syndrome (visceral fat area more than 100 cm<sup>2</sup>).

Another study in diet-induced overweight and obese subjects was conducted by providing subjects with 2-g oolong tea bag, 4 bags a day for 6 weeks [32]. Oolong tea bag was brewed by adding 300 mL of boiling water and steeped for 5 min before intake. Subjects consumed oolong tea twice in the morning and twice in the afternoon. Brewed tea was found to have OTPPs 33.65 mg/100 mL. The results showed that oolong tea ingestion slightly improved diet-induced obesity by lowering body weight in both women and men but the effect was more effective in women. Oolong tea consumption lowered serum triglyceride levels by 20% in subjects with hypertriglyceridemia and lowered plasma total cholesterol level by 8% in subjects with hypercholesterolemia.

According to all the published data on oolong tea and OTPPs, it indicates that oolong tea and OTPPs consumption have the beneficial effects on dyslipidemia.

### Possible mechanisms of oolong tea against dyslipidemia

Several studies report the possible mechanisms of oolong tea and OTPPs that modulate lipid profile. The first proposed mechanism mentioned is inhibitory effects against pancreatic lipase. Pancreatic lipase is the key enzyme in lipid digestion and absorption. It is well known that dietary fat is not able to be absorbed into intestine unless it has been hydrolyzed by pancreatic lipase [33]. It is one of the targets of obesity treatment by lowering lipid absorption.

The first study on inhibitory activity of water extract of oolong tea was shown by Han, et al. [34]. They reported that water extract of oolong tea inhibited pancreatic lipase at the concentrations of 500 – 2000 µg/mL, in a dose-dependent manner. Inhibitory activity against pancreatic lipase of oolong tea extract was shown again with  $IC_{50} = 0.94$  µg/mL [32]. In this study, catechins from oolong tea were also studied and the result showed that EGCG and ECG were the strongest catechins ( $IC_{50} = 0.16$  and 0.14, respectively). As I mentioned above that OTPPs are the key compounds in oolong tea that modulate lipid profile. Nakai, et al. (26) reported its inhibitory activity of OTPPs against pancreatic lipase, including primary polyphenols (flavan-3-ols, proanthocyanidins, chalcane-flavan dimers, oolonghomobisflavans and hydrolyzable tannins) and secondary polyphenols (compounds derived from primary oxidases during self-fermentation, including theasinensins and theaflavins). The results showed that flavan-3-ol gallate esters are the major compounds contributing to the inhibitory effects of oolong tea against pancreatic lipase and these effects were higher than those of EGCG and GCG. In this study, they also fractionated OTPPs from oolong tea by reversed-phase HPLC and tested its activity. The result showed that OTPPs had stronger activity than oolong tea extract.

The next proposed mechanism is reduction of intestinal triglyceride absorption. Lymphatic recovery of triglyceride was studied in rats cannulated with a vinyl tube at the left thoracic lymphatic duct [21]. Rats were administered with the test emulsion containing sodium taurocholate, fatty acid-free bovine serum albumin and triolein, followed by either oolong tea extract, OTPPs or caffeine. The results showed that oolong tea extract significantly reduced lymphatic absorption of triglyceride by 22% during the initial 5h after the treatment. OTPPs lowered lymphatic absorption of

triglyceride by 26% at 20 mg/head, which accounted for more than 60% of the corresponding dosage for the lymphatic absorption-lowering effects of oolong tea (200 mg/head). However this effect was not found in caffeine treatment with a dose corresponding to oolong tea extract. This suggested that OTPPs was the key component in oolong tea extract that lower lymphatic absorption of triglyceride and this contributes to the hypolipidemic effect of OTPPs from oolong tea. In the same study, mice given olive oil with OTPPs also had the lower elevation of plasma triglyceride compare to mice not given OTPPs. This confirmed that OTPPs suppressed postprandial hypertriglyceridemia and this mechanism was also confirmed with the human study as mentioned above [27].

Activity of oolong tea and OTPPs on inhibition of lipid digestion and absorption and lowering the lymphatic absorption of triglyceride was confirmed with the increased lipid excretion in feces. A double-blind, placebo-controlled crossover study confirmed this mechanism by showing the increase of fecal lipid excretion after the consumption of polyphenol-enriched oolong tea [28]. In that study, the participants were given 38 g of lipids from potato chips and total 750 mL of beverage, either polyphenol-enriched oolong tea or placebo at three meals for 10 days. The results showed that lipid excretion in the feces were significantly higher in the polyphenol-enriched oolong tea by 105% compared to placebo group. Additionally, the excretion of cholesterol into the feces tended to increase in the treatment group.

Increase of energy metabolism is another proposed mechanism of oolong tea and OTPPs that explain how they could reduce body weight and modulate lipid profile. A randomized crossover study performing with normal weight Japanese men reported that 24-h energy expenditure of men receiving oolong tea [35]. In this study, subjects were given either water, full-strength tea (15 g of tea in 1500 mL/day), half-strength tea (7.5 g of tea in 1500 mL/day) or water containing 270 mg caffeine, equivalent to the concentration in the full-strength tea treatment. The results showed that full-strength tea increased 24-h energy expenditure 2.9% and caffeine beverage increased 3.4% compared to water treatment. They also showed that fat oxidation rate of subjects receiving full-strength tea significantly increased by 12% compared to group receiving water. A study in healthy volunteers given brewed oolong tea found that consumption of oolong tea

containing OTPPs (68 mg/300 mL) immediately increased energy expenditure (at 30 min) and gradually increased up to 90 min and maintained until 120 min [36]. Green tea was also tested in the same study and the result showed that the cumulative increase of energy expenditure after the consumption of oolong tea is much higher than green tea. Although green tea contained double concentrations of caffeine and EGCG compared to oolong tea and oolong tea contained OTPPs much higher than green tea, this suggested that OTPPs from oolong tea played a crucial role in the increased energy expenditure of oolong tea.

As above mentioned about the beneficial effects of oolong tea and OTPPs on dyslipidemia, several mechanisms were proposed to elucidate these beneficial effects. Inhibition of pancreatic lipase and lowering lymphatic absorption of triglycerides in small intestine may be the major and direct mechanisms in lipid modulation because OTPPs would work before the lipid get absorbed into small intestine. These mechanisms are confirmed with the increase of lipid excretion in the feces of animals. Indirectly, OTPPs also increase energy expenditure, which could modulate the cellular lipids. With these all mechanisms, it indicates that OTPPs from oolong tea may be an alternative approach for lipid modulation in obese people.

## CONCLUSION

Nowadays, a number of people throughout the world are facing obesity. Obesity is the major cause of dyslipidemia that could lead to other chronic diseases such as cardiovascular disease and kidney disease, which could carry an enormous potential economic burden. Many pharmacological methods have been developed to treat dyslipidemia and related symptoms. However, these treatments are costly and usually come with adverse side effects. The development of dietary agents for the prevention or treatment of dyslipidemia alone or in combination with lifestyle changes and pharmaceutical agents, could be a cost-effective and safe approach to this problem.

Oolong tea is one of the beverages showing beneficial effects on dyslipidemia by modulation lipid profile. Many researchers have carried studies on oolong tea polymerized polyphenols (OTPPs) which is the unique compound in oolong tea and it is proposed to be the active compound in oolong tea. Collectively, the studies described in this review provide compelling but experimental evidence of a

potential of OTPPs and oolong tea in dyslipidemia. No report contradicting this evidence was found in the scientific literature. A common weakness of all the human studies reviewed herein was the studies were conducted in a short term. There is only one case-control study that showed the association between oolong tea consumption and the risk factor of dyslipidemia.

Taken all the studies together, I could summarize that oolong tea polymerized polyphenols in oolong tea has the potential health benefit on dyslipidemia. OTPPs could lower serum triglycerides, total cholesterol and LDL cholesterol despite no effects on HDL cholesterol. The possible mechanisms of OTPPs are inhibitory effects against pancreatic lipase, reduction of lymphatic triglyceride absorption in small intestine and increase of energy expenditure. Although oolong tea has a long history of safe use in the diet, no controlled animal and human studies have been conducted to determine the maximum tolerated dose of oolong tea or OTPPs. Therefore large scale controlled human intervention studies would be needed to confirm the published data and it could confirm that oolong tea and OTPPs would not have adverse effects.

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