EFFECTS OF ASTAXANTHIN SUPPLEMENTATION ON BIOLOGICAL PARAMETERS IN THAI HEALTHY VOLUNTEERS

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ผลของการเสริมแอสตาแซนธินต่อพารามิเตอร์ทางชีวภาพในอาสาสมัครชาวไทยที่มีสุขภาพดี

น.ส.ชื่นใจ สระทองแฝง

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ชื่นใจ สระทองแฝง : ผลของการเสริมแอสตาแซนธินต่อพารามิเตอร์ทางชีวภาพในอาสาสมัครชาวไทยที่มีสุขภาพดี. (EFFECTS OF ASTAXANTHIN SUPPLEMENTATION ON BIOLOGICAL PARAMETERS IN THAI HEALTHY VOLUNTEERS) อ.ที่ปรึกษาหลัก : รศ. ภญ. ดร.กุลวรา เมฆสวรรค์, อ.ที่ปรึกษาร่วม : ศ. ภก. ดร.ปิติ จันทร์วรโชติ

แอสตาแซนธินเป็นสารต้านออกซิเดชันที่มีการนำมาใช้ในรูปแบบผลิตภัณฑ์เสริมอาหารกันอย่างแพร่หลาย เพื่อประโยชน์ต่อสุขภาพ อย่างไรก็ตาม ยังมีข้อมูลไม่เพียงพอ เกี่ยวกับผลของการเสริมแอสตาแซนธินในระยะยาว ต่อพารามิเตอร์ทางชีวภาพทั่วไป ของผู้ที่มีสุขภาพดี การศึกษานี้จึงมีวัตถุประสงค์ เพื่อศึกษาผลของการเสริมแอสตาแซนธินเป็นระยะเวลา 12 สัปดาห์ ต่อระดับน้ำตาลในเลือด ระดับไขมัน และพารามิเตอร์ด้านโลหิตวิทยาในอาสาสมัครที่มีสุขภาพดี มีผู้เข้าร่วมการศึกษาทั้งหมด 33 คน (16 คน ในกลุ่มทดลอง และ 17 คนในกลุ่มยาหลอก) ผู้เข้าร่วมการศึกษาในกลุ่มทดลอง ได้รับการเสริมแอสตาแซนธินวันละ 4 มิลลิกรัม ในขณะที่กลุ่มยาหลอกได้รับเม็ดแคปซูลน้ำมันถั่วเหลือง เป็นระยะเวลานาน 12 สัปดาห์ โดยก่อนและหลังการเสริมผลิตภัณฑ์ มีการเก็บข้อมูลดัชนีมวลกาย ความดันโลหิต และมีการเก็บตัวอย่างเลือด เพื่อตรวจสอบระดับแอสตาแซธินในเลือด ระดับน้ำตาลในเลือดหลังอดอาหาร ระดับไขมัน และพารามิเตอร์ด้านโลหิตวิทยา นอกจากนี้ยังมีการประเมินการรับประทานอาหารโดยใช้แบบบันทึกการบริโภคอาหาร 3 วัน และมีการศึกษาผลไม่พึงประสงค์ในระหว่างการเสริมผลิตภัณฑ์ด้วย หลังการเสริมแอสตาแซนธิน พบว่าระดับแอสตาแซนธินในเลือดเพิ่มขึ้นอย่างมีนัยสำคัญ ($p < 0.05$) และระดับแอสตาแซนธินมีค่าสูงกว่ากลุ่มที่ได้รับยาหลอกอย่างมีนัยสำคัญถึง 5 เท่า ($p < 0.05$) ไม่พบการเปลี่ยนแปลงของระดับน้ำตาลในเลือดหลังอดอาหาร คอเลสเตอรอลรวม เอชดีแอลคอเลสเตอรอล และแอลดีแอลคอเลสเตอรอล หลังจากได้รับการเสริมด้วยแอสตาแซนธินเป็นเวลา 12 สัปดาห์ แม้พบว่า กลุ่มที่ได้รับแอสตาแซนธินมีระดับไตรกลีเซอไรด์เพิ่มขึ้นอย่างมีนัยสำคัญ ($p < 0.05$) ในสัปดาห์ที่ 12 แต่ระดับไตรกลีเซอไรด์ที่เพิ่มขึ้นนั้นยังอยู่ในระดับปกติ ไม่พบความแตกต่างระหว่างกลุ่ม ของระดับน้ำตาลในเลือดหลังอดอาหารและระดับไขมัน ในสัปดาห์ที่ 12 ในการศึกษานี้ การเสริมแอสตาแซนธินไม่มีผลต่อพารามิเตอร์ด้านโลหิตวิทยาส่วนใหญ่ ผลของการศึกษาแสดงให้เห็นว่า ผู้เข้าร่วมการศึกษาในกลุ่มทดลอง ได้รับพลังงานรวมและคาร์โบไฮเดรตลดลงอย่างมีนัยสำคัญ ในสัปดาห์ที่ 12 ($p < 0.05$) และมีการบริโภคโซเดียมต่ำกว่ากลุ่มที่ได้รับยาหลอกอย่างมีนัยสำคัญ ($p < 0.05$) ไม่พบผลข้างเคียงที่รุนแรงจากการเสริมแอสตาแซนธิน โดยพบอุจจาระมีสีแดง มีอาการคันเล็กน้อย และความอยากอาหารลดลง ซึ่งอาการเหล่านี้ค่อย ๆ ดีขึ้นเอง โดยไม่ต้องหยุดการเสริมแอสตาแซนธิน ผลการศึกษานี้แสดงให้เห็นว่าการเสริมแอสตาแซนธินขนาด 4 มิลลิกรัมต่อวัน เป็นเวลา 12 สัปดาห์ ไม่มีผลต่อระดับน้ำตาลในเลือด ระดับไขมัน รวมไปถึงพารามิเตอร์ด้านโลหิตวิทยา และอาจมีผลลดการบริโภคอาหารในผู้ที่มีสุขภาพดี

สาขาวิชา	อาหารเคมีและโภชนศาสตร์ทางการแพทย์	ลายมือชื่อนิสิต .....
ปีการศึกษา	2562	ลายมือชื่อ อ.ที่ปรึกษาหลัก .....
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Chuenjai Sratongfaeng : EFFECTS OF ASTAXANTHIN SUPPLEMENTATION ON BIOLOGICAL PARAMETERS IN THAI HEALTHY VOLUNTEERS. Advisor: Assoc. Prof. KULWARA MEKSAWAN, Ph.D. Co-advisor: Prof. PITHI CHANVORACHOTE, Ph.D.

Astaxanthin, a powerful antioxidant, is widely used as a dietary supplement to promote several health benefits. However, the effects of long-term supplementation on general biological parameters in healthy adults is still insufficient. The objective of this study was to investigate the effects of 12-week astaxanthin supplementation on blood glucose, lipid profile and hematological parameters in healthy volunteers. Thirty-three healthy participants (16 in the experimental group and 17 in the placebo group) were enrolled in the study. The participants in the experimental group were supplemented with 4 mg/day of astaxanthin while those in the placebo group were supplemented with soybean oil capsule for 12 weeks. Body mass index and blood pressure were recorded, and blood sample was collected to determine serum astaxanthin, fasting blood glucose (FBG), lipid parameters, and hematological parameters before and after the supplementation. Moreover, the daily dietary intake was assessed by 3-day food record, and adverse effects during supplementation were investigated. After astaxanthin supplementation, serum astaxanthin concentration significantly increased from baseline ($p < 0.05$), and significantly 5-fold higher than those in the placebo group ($p < 0.05$). There were no significant changes in FBG, total cholesterol, high-density lipoprotein cholesterol, and low-density lipoprotein cholesterol from baseline after 12-week supplementation in both groups. Although significant increased triglyceride level was found in the experimental group at week 12 ($p < 0.05$), the level was still in the reference range. No differences in FBG and lipid parameters at week 12 between groups were observed. In this study, most of hematological parameters were not affected by astaxanthin supplementation. Interestingly, the results showed reduced total energy and carbohydrate intakes of the participants in the experimental group at week 12 ($p < 0.05$), and their sodium intake was significantly lower than those in the placebo group ($p < 0.05$). No serious adverse effects were reported. Red stool, mild itching and loosing appetite occurred and then became better without discontinuing the supplementation. The findings suggested no effects on general biological parameters and potential dietary intake-lowering effect of astaxanthin in healthy individuals.

Field of Study:	Food Chemistry and Medical Nutrition	Student's Signature .....
Academic Year:	2019	Advisor's Signature .....
		Co-advisor's Signature .....

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LIST OF ABBREVIATIONS

LD_{50}	50% lethal dose
°C	degree celsius
µg	microgram
µL	microlitre
µm	micrometre
µmol/L	micromole per litre
ATP	adult treatment panel
AUC	area under the curve
BMI	body mass index
CBC	complete blood count
CHO	carbohydrate
CVD	cardiovascular disease
CYP450	cytochrome P450
DNA	Deoxyribonucleic acid
EFSA	European food safety authority
FBG	fasting blood glucose
fL	femtoliters
g	gram
g	gravity
g/dL	grams per decilitre
g/kg	gram per kilogram
H. pluvialis	*Haematococcus pluvialis*
HDL-C	high density lipoprotein cholesterol
HOMA-IR	homeostatic model assessment of insulin resistance
HPLC	high performance liquid chromatography
kcal	kilocalorie
kg	kilogram

kg/m^2	kilogram per square metre
LDL-C	low density lipoprotein cholesterol
mg	milligram
mg/dL	milligrams per decilitre
mg/kg	milligram per kilogram
mg/kg/day	milligram per kilogram per day
mg/L	milligram per Litre
mmHg	a millimetre of mercury
NCEP	the national cholesterol education program
NF-kB	nuclear factor-kappa B
ng/mL	nanogram per millilitre
nm	nanometre
NOAEL	no observed adverse effect levels
pg	picogram
PPAR	peroxisome proliferator activated receptor
ppm	parts per million
PTECs	proximal tubular epithelial cells
ROS	reactive oxygen species
RP-HPLC	reversed-phase high performance liquid chromatography
TC	total cholesterol
TNF	tumor necrosis factor
USD/kg	United States dollar per kilogram
UV	ultraviolet
yr	year

CHAPTER I

INTRODUCTION

Background and rationale

Astaxanthin, a group of xanthophyll carotenoids, is usually found in various plants, microalgae (especially *Haematococcus pluvialis* which is known as the highest source of astaxanthin), and aquatic animals such as shrimp, crab, and salmon (Ambati et al., 2014). It is generally existed as wide ranges of pigments such as red, orange and yellow (Eldahshan and Singab, 2013). Other than giving colorful pigments, astaxanthin is a powerful antioxidant that can effectively scavenge free radicals since its molecular structure consists of many conjugated double bonds (Satoh, 2016). These conjugated double bonds can absorb electrons and form adducts with oxygen species (Bonner and Arbiser, 2014). Additionally, astaxanthin transmembrane orientation provides an exposure to both hydrophilic and hydrophobic parts of cellular membranes. For this reason, astaxanthin possesses stronger antioxidant properties than other carotenoids in preventing lipid peroxidation (Leopold, 2015; Pashkow et al., 2008). Consequently, astaxanthin supplementation has been purposed to prevent several health complications such as skin deterioration, cardiovascular disease, liver disease, neurodegenerative disease as well as eye problems (Guerin et al., 2003).

According to toxicological studies, astaxanthin has been asserted for safety in animal over the past decades. As stated by the previous study of astaxanthin subchronic toxicity in Wistar rats for 90 days, the oral LD_{50} of astaxanthin biomass was higher than 12 g/kg of body weight. The no-observed-adverse-effect levels (NOAEL) were 465 and 557 mg/kg of body weight in male and female rats, respectively. The study recommended the proper doses of astaxanthin supplementation in human around 2 to 6 mg/kg/day which were lower than the

suggested NOAEL (Stewart et al., 2008). In addition, astaxanthin was administered for the purpose of lowering blood pressure and improving insulin sensitivity in Sprague-Dawley rats. The eight months administration of 100 mg/kg of astaxanthin showed positive effects without toxicities on blood biochemistry and animal behavior (Preuss et al., 2011). Another safety assessment of astaxanthin indicated that the 13-week ingestion of astaxanthin for up to 700-920 mg/kg/day did not produce any significant dose-related abnormalities on clinical pathology, animal behavior, reproductive parameters, and tissue examinations (Buesen et al., 2015).

In humans, there were many studies of astaxanthin efficacy in both healthy and unhealthy individuals. In type 2 diabetes, 8 mg of astaxanthin supplementation for 8 weeks improved glucose metabolism and blood pressure. No serious adverse events were observed (Mashhadi et al., 2018). In individuals with mild hyperlipidemia, variable doses of astaxanthin supplementation for 12 weeks revealed such positive effects on lipid profile that was, 6 and 12 mg of astaxanthin improved high density lipoprotein cholesterol (HDL-C) levels (Yoshida et al., 2010). In 27 Korean overweight adults, 12-week administration of astaxanthin for 20 mg/day decreased low density lipoprotein cholesterol (LDL-C) levels. A few adverse effects including gastrointestinal events, reddish fecal color and increased bowel movements were reported (Choi et al., 2011b).

In healthy individuals, the efficacy of astaxanthin on lipid peroxidation was published. Thirty-nine healthy non-smoking Finnish males who were treated with 8 mg/day of astaxanthin supplementation for 12 weeks showed decreased in 12-and 15-hydroxy fatty acids. However, there were no alteration in lipid profile (Karppi et al., 2007). For astaxanthin safety aspects, a study in 35 healthy adults who were treated with 6 mg of astaxanthin extracted from *Haematococcus pluvialis* once daily for 8 weeks showed no alteration in hematological parameters, blood glucose levels, kidney function, and blood pressure (Spiller and Dewell, 2003).

Regarding to the previous studies, astaxanthin efficacy was frequently investigated in people with health complications, and the duration was typically around 12 weeks (Choi et al., 2011b; Mashhadi et al., 2018; Yoshida et al., 2010). The study of astaxanthin safety aspects were still limited. Even if the study was conducted, it was a short-period study (Spiller and Dewell, 2003). Moreover, most of the previous studies (Choi, Youn, and Shin, 2011; Nakagawa et al., 2011; Satoh et al., 2009; Yoshida et al., 2010) were conducted with high-dose astaxanthin treatment, ranging from 12 to 20 mg/day. Therefore, the study with lower dose of astaxanthin still need more concern in the safety aspects. In fact, EFSA (2014) recommended a maximum consumption of astaxanthin from the novel food ingredients for up to 4 mg/day, and most studies presented the beneficial results from a daily intake of 4 mg (Seabra and Pedrosa, 2010). Djordjevic et al. (2012) and Baralic et al. (2013) showed the positive effects of 4 mg astaxanthin for 12 weeks in healthy individuals. However, blood astaxanthin concentration was not presented, and adverse effects were not mentioned. In Thailand, Food and Drug Administration, (2017) announced that astaxanthin from *Haematococcus pluvialis* was approved as dietary supplement at the maximum dose of 6 mg/day.

The conceptual framework of this present study is presented in **Figure 1.** To study the safety of low dose astaxanthin that comply with Thai FDA recommendation in Thais, therefore, this study aimed to investigate the effects of 4-mg astaxanthin supplementation for 12 weeks on general biological parameters including lipid profile, blood glucose and hematological parameters in Thai healthy volunteers.

Objective of the study

To investigate the effects of astaxanthin supplementation for 12 weeks on lipid parameters, blood glucose levels and hematological parameters in Thai healthy volunteers

Benefits of the study

The results obtained in this study provides the knowledge on the safety of astaxanthin in humans, and the data are also helpful in providing a suitable recommendation to healthy people who require astaxanthin supplementation for the purpose of improving health status.

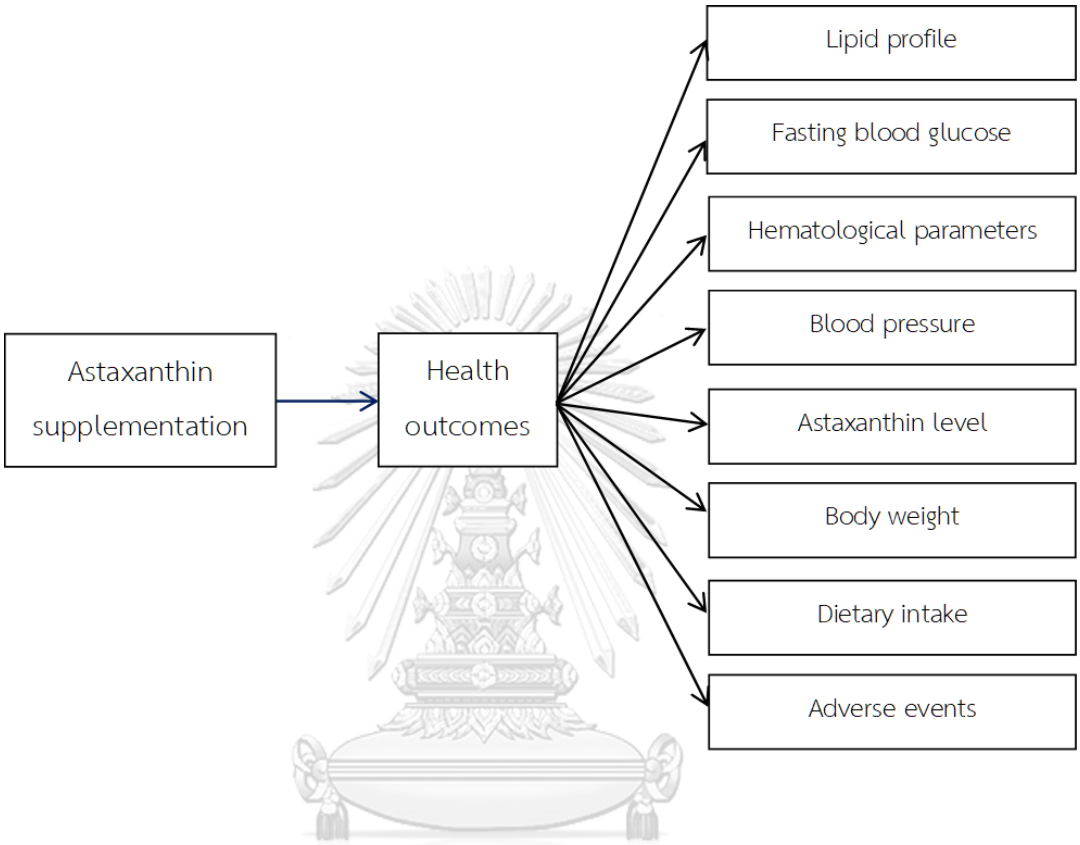

Figure 1 Conceptual framework of the present study

CHAPTER II

LITERATURE REVIEW

2.1 Astaxanthin

2.1.1 Chemical structure and sources

Astaxanthin is a natural occurring lipid soluble red pigment belongs to xanthophyll carotenoids. Chemical formula of astaxanthin is $C_{40}H_{52}O_4$. The chemical structure consists of conjugated double bond connected two oxygenated beta-ionone-type rings together as presented in **Figure 2**. This structure allows astaxanthin to absorb UV light at wavelength ranging from 400 to 500 nm (Molino et al., 2018). Since two stereogenic carbons (3 and 3′) exist on the beta-ionone moieties, astaxanthin can be found in three forms: two chiral [(3S, 3′S) and (3R, 3′R)] and one meso (3R, 3′S). The 3S, 3′S is the most commonly found in natural sources. For example, red yeast *Phaffia rhodozyma*, microalgae *Haematococcus pluvialis,* and aquatic animal such as salmon, shrimp, and crab ((Polotow et al., 2014; Visioli and Artaria, 2017; Wang et al., 2014). The approximated quantity of astaxanthin in natural source is presented in **Table 1.**

Currently, several evidences have noted that *H. pluvialis* is the greatest source of astaxanthin manufacturing. In freshwater, *H. pluvialis* is the unicellular green microalgae. When growing under stress condition that is, nitrogen limitation, increased light intensity, sulfate or phosphate starvation, and higher saline levels, *H. pluvialis* undergoes cell division and begins to produce red carotenoid pigment also called astaxanthin (Boussiba, 2000; Shah et al., 2016). The recent experimental finding has indicated that the extraction of astaxanthin from *H. pluvialis* can be performed by using organic solvents which give astaxanthin yield up to 17 mg/g of dry biomass. Extracted astaxanthin from red yeast and microalgae costs around 2,500-7,000 USD/kg. The valuable of astaxanthin is probably increasing to 700 million euro in the next three years (Koller et al., 2014; Molino et al., 2018).

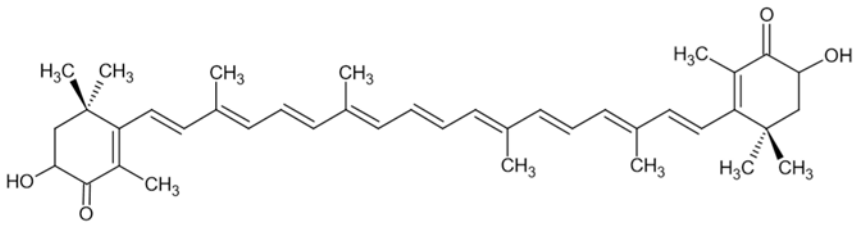

Figure 2 Chemical structure of astaxanthin (Visioli and Artaria, 2017)

Table 1 The approximated quantity of astaxanthin (Ambati et al., 2014; Seabra and Pedrosa, 2010)

Sources	Quantity
Haematococcus pluvialis	2.7 - 3.8 g/100 g dry mass
Phaffia rhodozyma	0.5 g/100 g dry mass
Wild salmon	2.6 - 2.8 mg/100 g flesh
Shrimp	1.41 - 1.69 mg/100 g flesh
Farmed salmon	0.6 - 0.8 mg/100 g flesh

2.1.2 Pharmacokinetics

As astaxanthin is a lipid soluble pigment, its absorption is comparable to those foods containing fat (Fiedor and Burda, 2014). Briefly, astaxanthin is incorporated into mixed micelles in intestinal lumen, and the micelles are taken up into mucosal cells via passive diffusion. Then, astaxanthin is united into chylomicrons followed by releasing into lymphatic system. When astaxanthin travels to the liver in chylomicron form, the chylomicron is digested by lipoprotein lipase leaving astaxanthin to bind to lipoproteins, particularly LDL-C, to further distribute to other organs and tissues. The examples of major accumulated sites for astaxanthin are liver, adrenal gland, adipose tissue, retina, testes, and skin. Furthermore, due to lipid soluble properties of astaxanthin, dietary containing fat can effectively enhance astaxanthin absorption from gastrointestinal tract. Other factors known to influence carotenoid absorption including age, gender, pharmaceutical dosage form, and smoking status (Parker, 1996; Visioli and Artaria, 2017). Some evidences have suggested that people who smoke tend to have shorter elimination half-life of serum astaxanthin (Okada et al., 2009).

The studies of astaxanthin pharmacokinetics have also conducted for more than two decades. In animals, male Wistar rats were treated with foods containing 300 mg/kg of astaxanthin for four days. Hepatocytes were cultured and analyzed for astaxanthin metabolism. The results suggested that cytochrome P450 (CYP450) were not involved in the metabolism of astaxanthin in rats (Wolz et al., 1999). In contrast, Choi et al., (2011a) suggested that astaxanthin was predominantly metabolized by CYP1A1 and CYP1A2 followed the oral administration of 100 and 200 mg/kg of astaxanthin in male Sprague-Dawley rats. The administration of astaxanthin via oral route exhibited dose-independent pharmacokinetics since the plasma concentration of astaxanthin at the dose of 100 mg/kg were comparable to that dose of 200 mg/kg. In addition, the gastrointestinal absorption of astaxanthin obeyed flip-flop model. The flip-flop model explains the situation when absorption rate of a compound is

slower than elimination rate. Therefore, the compound persists in the body depending on its absorption rather than elimination process (Gupta, 2016).

In humans, astaxanthin capability to induce cytochrome P450 genes was investigated in hepatocytes. The hepatocytes were obtained from patients who underwent a partial hepatectomy. The results revealed that astaxanthin acted as CYP3A4 and CYP2B6 inducer. Additionally, after a single dose of 100 mg astaxanthin were administered, the metabolites were detected. That is, 3-hydroxy-4-oxo-beta-ionol and 3-hydroxy-4-oxo-beta-ionone. These metabolites were conjugated and reduced to 3-hydroxy-4-oxo-7, 8-dihydro-beta-ionol and 3-hydroxy-4-oxo-7, 8-dihydro-beta-ionone (Choi et al., 2011a; Kistler et al., 2002).

Additional information relating to astaxanthin pharmacokinetics in human were also published. Time to peak and elimination half-life of astaxanthin seem to vary in different studies. A single dose of 100-mg astaxanthin was orally administered in three men aged 37-43 years. Time to peak of astaxanthin was 6.7 ± 1.2 hours, and maximum concentration of astaxanthin was 1.30 ± 0.10 mg/L. The plasma elimination half-life of astaxanthin was 21 ± 11 hours (Østerlie et al., 2000). In another study, a single dose of 10 and 100 mg of astaxanthin were orally administered in three men aged 41-50 years four weeks apart. The results showed time to peak was 11.50 hours, and the maximum concentration of astaxanthin was 0.08 and 0.28 mg/L after administered 10 and 100 mg of astaxanthin, respectively. The plasma elimination half-life of astaxanthin in this study was 52 ± 40 hours, and the astaxanthin dose response was non-linear (Coral-Hinostroza et al., 2004).

The study conducted by Ruiz-Núñez et al., (2014) presented the half-life of astaxanthin for 18 hours after oral administration of a single dose of astaxanthin for 40 mg in 4 healthy adults. In addition, during the maintenance dose of 8 mg astaxanthin for 17 days, plasma astaxanthin gradually increased until day 10. Then, the plasma astaxanthin slowly declined to a steady state concentration similar to

that after day 2. Correspondingly, Ruiz-Núñez et al., (2014) suggested the daily administration of astaxanthin should be taken. At least, in an early period when the total body equilibrium has not been reached yet. The study in Asian population found that smoking was the factor affecting astaxanthin elimination half-life. A single dose of 48-mg astaxanthin was orally administered in smoker and non-smoker participants, before and after meal. The results showed higher bioavailability in after meal group compared to before meal group, and smoking significantly decreased elimination half-life of astaxanthin (Okada et al., 2009).

2.1.3 Toxicology

In acute toxicity study, oral LD_{50} of astaxanthin-rich biomass from *Haematococcus pluvialis* was higher than 12 g/kg of body weight in Sprague-Dawley rats. A short-term repeated-dose toxicity study revealed that 6 mg/kg/day for 14 consecutive days did not cause treatment-related deaths. No adverse effects and treatment-related alterations were observed. In subchronic toxicity study, the diets mixed with astaxanthin-rich biomass: 10,000 ppm, 50,000 ppm, and 200,000 ppm, were provided *ad libitum* to Wistar rats for three months. The results showed that plasma astaxanthin increased up to 190.20 ng/mL in rats treated with 200,000 ppm of astaxanthin-rich biomass. No serious adverse effects were observed, except for fur and feces discoloration in all groups exposed to astaxanthin. Based on the results of subchronic toxicity study, the no-observed-adverse-effect-level (NOAEL) of astaxanthin was set at 465 and 557 mg/kg/day in male and female rats, respectively. Thus, the recommended dose of astaxanthin as dietary supplement in humans was supposed to be 2 to 6 mg/day (around 0.1 mg/kg/day in adults weighing 60 kg). The recommended dose was 800 folds lesser than the NOAEL (Stewart et al., 2008).

According to European Food Safety Authority (EFSA), astaxanthin has no genotoxicity and low allergenicity effects based on the available of *in vitro* and *in vivo* studies. Additionally, astaxanthin lacks of pro-oxidant and vitamin A precursor

activity when compared to beta-carotene. Therefore, adding astaxanthin as food ingredients at the level of proposed use may possibly not increase the risk of lung cancer in cigarette smokers. EFSA also suggests that the recommended dose of astaxanthin as novel food ingredients should not be more than 4 mg/day which is 2-fold higher than the acceptable daily intake (EFSA Panel on Dietetic Products and Allergies, 2014).

2.2 Health benefits of astaxanthin

2.2.1 Antioxidant activity

Living in aerobic atmosphere is the main reason for humans to get expose to a variety of reactive oxygen species (ROS) or nitrogen species which possibly cause cellular damage (both cellular function and structure) such as DNA, proteins, lipids, and carbohydrates (Visioli and Artaria, 2017). The ROS, for example, hydroxyl radical, peroxide ion, and superoxide radical were generated under physiological states (metabolism pathways and electron transporter chain), or when exposure to UV-light, heavy metals, and pollutants. As a result, an imbalance between prooxidant and antioxidant, also called oxidative stress, leads to several illness in humans (Sinha et al., 2013). Therefore, the study of antioxidant effect of astaxanthin has become an important aspect in recent years.

Astaxanthin has antioxidant effects in both direct and indirect actions (Visioli and Artaria, 2017). For in direct action, astaxanthin acts against ROS by scavenging singlet oxygen and peroxyl radicals. This mechanism was investigated by measuring free radical actions using electron spin resonance and spin trapping methods (Dose et al., 2016; Visioli and Artaria, 2017). Moreover, another hypothesis for direct action of astaxanthin to ROS is related to its transmembrane alignment (**Figure 3**). Astaxanthin can penetrate lipid bilayer of cellular membranes and expose to both hydrophilic and hydrophobic parts. As a result, when compare to other antioxidants that locate only in specific parts of cellular membranes such as vitamin C or vitamin E, astaxanthin effectively scavenges more ROS than those antioxidants (Pashkow et al., 2008).

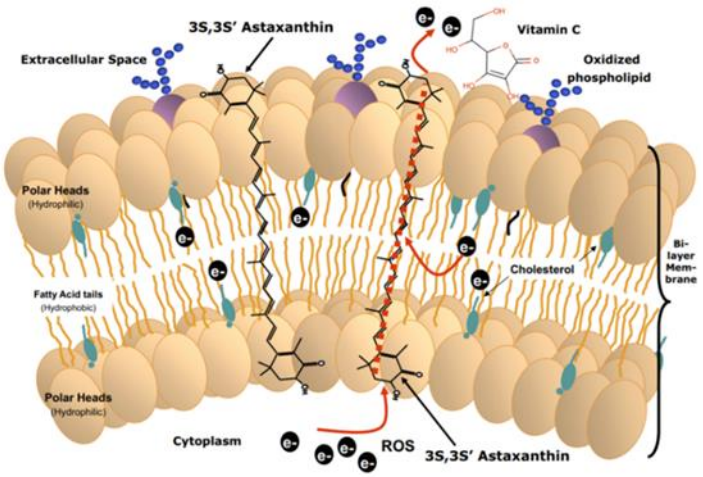

Figure 3 Astaxanthin transmembrane alignment (Pashkow et al., 2008)

Besides direct action, astaxanthin also exhibits its indirect action via enhancing antioxidant enzyme activities. Owning to the fact that humans have naturally occurring physiological systems to defense ROS mediated cellular damage, especially antioxidant enzymes such as catalase, superoxide dismutase, glutathione peroxidase, glutathione reductase, and paraoxonases (Matés and Sánchez-Jiménez, 1999). These enzymatic antioxidants deal with ROS by converting highly reactive radicals to the less reactive species. For example, catalase reacts with hydrogen peroxide to form water and oxygen. Superoxide dismutase converts superoxide radical to less reactive hydrogen peroxide (Matés et al., 1999). The study indicated that astaxanthin improved paraoxonases-1 activity in young soccer players by protecting thiol groups against oxidative alteration after they were supplemented by 4 mg of astaxanthin for 12 weeks (Baralic et al., 2013). Another *in vitro* study demonstrated the greater glutathione peroxidase activity in white blood cells treated with astaxanthin (Macedo et al., 2010).

2.2.2 Anti-inflammatory activity

Role of astaxanthin in reducing inflammatory markers in cell lines has been revealed. The study investigated the antioxidant effects of shrimp waste products which was believed to compose of astaxanthin on rat alveolar macrophages. The alveolar obtained from male and female Wistar rats were cultured then treated with 43.50 μg/mL shrimp waste extract. The results revealed that shrimp waste extract significantly lowered TNF-alpha levels (Santos et al., 2012). Since TNF-alpha acts as macrophage activator and cytokine stimulator, therefore, astaxanthin from shrimp waste products might have benefits for individuals who suffer from the antioxidant imbalance conditions. Additionally, the study in proximal tubular epithelial cells (PTECs) found that, under high glucose exposure, PTECs treated with astaxanthin showed low levels of total ROS generation and thiobarbituric acid reactive substances. Moreover, inducible nitric oxide synthase, cyclooxygenase-2, and nuclear factor-kappa B (NF-kB) nuclear translocation in PTECs were also modulated by astaxanthin. This can be attributed to its ROS-attenuating properties (Kim et al., 2009).

In humans, the study of anti-inflammatory effects of astaxanthin is still limited. The available data indicate that 2 and 8 mg of astaxanthin supplementation for 8 weeks resulting in adaptive immune system improving, both humoral and cell-mediated immune responses. Astaxanthin significantly amplified natural killer cell cytotoxic activity, T cell and B cell mitogen-induced lymphocyte proliferation, production of interleukin 6 and interferon-gamma, and lymphocyte function-associated antigen 1 expression. Additionally, astaxanthin can reduce plasma C-reactive protein and plasma 8-hydroxy-2'-deoxyguanosine, which is one of DNA damage biomarker (Park et al., 2010).

2.2.3 Antihyperglycemic activity

Diabetes is a chronic metabolic disorder resulting from either insulin deficiency or defects in insulin signaling, or both. Prolonged high blood glucose contributes to several diabetic complications. The benefits of astaxanthin in blood glucose reduction were suggested in animals and humans. According to the study conducted in Sprague-Dawley rats, 100 mg/kg of astaxanthin supplementation for two months significantly lowered homeostatic model assessment of insulin resistance (HOMA-IR), area under the curve (AUC) of glucose tolerance test, and circulating glucose levels when compared to control group (Preuss et al., 2011). In Mus musculus mice treated with high fructose, high fat diet, and 2 mg/kg of astaxanthin for two months, astaxanthin improved insulin sensitivity via two possible mechanisms: reducing serine phosphorylation of insulin receptor substrates proteins and enhance glucose metabolism by controlling metabolic enzymes (Bhuvaneswari and Anuradha, 2012). Moreover, *in vitro* study indicated that ROS was generated under high glucose concentration (Lin et al., 2005). Astaxanthin not only diminishes oxidative damage in human umbilical vein endothelial cells (HUVECs) but also inhibits c-Jun N-terminal kinase (JNK) and p38 phosphorylation which are responsive to inflammatory processes (Abdelzaher et al., 2016).

In human, the participants with type 2 diabetes who were treated with 8 mg of astaxanthin for eight weeks exhibited significantly lower fructosamine and plasma glucose concentration compared to placebo. The outcomes might relate to higher adiponectin concentration since adiponectin itself acts as insulin sensitizer, AMP-activated protein kinase stimulator and peroxisome proliferator activated receptor alpha (PPAR-alpha) activator in muscle and liver (Mashhadi et al., 2018).

2.2.4 Antidyslipidemic activity

Dyslipidemia is considered as the main modifiable risk factor contribute to cardiovascular diseases (CVD). Elevated blood lipid concentrations including TC, LDL-C and TG, or reduced HDL-C levels are the primary screening of abnormal lipid profile. Prolonged dyslipidemia can lead to amplified inflammatory adipokine levels, endothelial dysfunction, thrombus, and atherosclerotic plaques formation. Thus, improving lipid profile and increasing in antioxidant capacity may lower the chance of developing CVD (Chehade et al., 2013; Leopold, 2015).

Previous studies suggested the benefits of astaxanthin in lowering lipid levels. Mice fed with high-fat diet mixed with 0.03% of astaxanthin for 12 weeks exhibited reducing plasma triacylglycerol and increasing enzymes involved in peroxisomal beta-oxidation (Yang et al., 2014). Another study presented the positive effects of astaxanthin in obese mice. Female ddY mice fed with high-fat diet plus astaxanthin in varying doses: 1.2, 6, and 30 mg/kg/day for 60 days revealed lowering body weight in dose-dependent manner. Moreover, plasma TG, TC, and liver TG were significantly lowered in high dose group when compared to other groups. These outcomes support the evidence of astaxanthin in decreasing the chance of getting fatty liver and obese induced by high-fat diet (Ikeuchi et al., 2007).

In human, astaxanthin also showed the positive effects on lipid parameters. Non-obese with mild hyperlipidemia participants aged 20-60 years who were treated with 6, 12, and 18 mg/day of astaxanthin for up to 12 weeks gained benefits from astaxanthin supplementation. It was found that 12 and 18 mg/day of astaxanthin significantly reduced TG, and increased serum adiponectin while 6 and 12 mg/day significantly raised HDL-C levels (Yoshida et al., 2010). Another study conducted in overweight participants (body mass index more than 25 kg/m^2) aged 20-55 years showed the effects of 20 mg astaxanthin supplementation for 12 weeks. Lipid parameters including LDL-C, apolipoprotein B, and apolipoprotein B/apolipoprotein A1 ratio were improved when compared to baseline (Choi et al., 2011b).

2.2.5 Antihypertensive activity

Hypertension can be developed by a few causative factors including blood rheology, mostly via increasing in red blood cell aggregation, plasma viscosity, and total peripheral resistance. These may lead to cardiovascular risk development due to blood flow disturbances (Becker, 1993). The study of antihypertensive mechanism of astaxanthin reveal several related mechanisms in animals. According to Hussein et al. (2005), male spontaneously hypertensive rats were treated with 5 mg/kg/day of astaxanthin for 7 weeks had lower systolic and diastolic blood pressure when compared to control group. Astaxanthin also reduced aortic contraction induced by xanthine/xanthine oxidase and aortic contractile responses induced by phenylephrine and angiotensin II. The vasorelaxant response to sodium nitroprusside was found superior to control group. Additionally, astaxanthin could reduce blood transit time of rats in the treatment group. Nevertheless, astaxanthin showed no effects on blood cell counts and plasma fibrinogen levels (Hussein et al., 2005). Another study indicated that astaxanthin could lower systolic blood pressure as well as improve cardiac hypertrophy. In addition, astaxanthin also enhanced vascular bed relaxation via inducing nitric oxide bioavailability (Monroy-Ruiz et al., 2011). In human, the available data on antihypertensive activity of astaxanthin is still inconclusive. One study conducted in 20 adult men aged 57.5 ± 9.8 and 50.8 ± 13.1 years in placebo and treatment group, respectively. The results noted that supplementation with 6 mg/day of astaxanthin for ten days could significantly reduce whole blood transit time (Miyawaki et al., 2008).

The literature review of astaxanthin presented several health benefits in humans. Astaxanthin reduced oxidative stress by enhancing antioxidant defense mechanisms. Moreover, astaxanthin has been found to decrease risk of developing chronic diseases by enhancing lipid profile and fasting blood glucose, as well as reducing blood pressure. Although information on health benefits and safety in healthy individuals are still limited, astaxanthin supplementation did not seem to cause any serious adverse events. Thus, astaxanthin may be safely used as a supplement to promote positive effects on health outcomes of healthy individuals.

CHAPTER III

MATERIALS AND METHODS

3.1 Participants

Healthy males and females aged 20-60 years were recruited, and health examination was conducted as a primary screening. The inclusion criteria were healthy adults with BMI in the range of 18.50-24.90 kg/m^2 who were able to take dietary supplements in dosage form of soft gelatin capsules. All participants had no history of astaxanthin supplementation for at least 3 months prior to enroll in this study. The participants had no experience of acute or chronic infection as well as receiving any surgery for at least 1 month prior to enrollment. In addition, they must be able to read, write, and understand Thai language.

The participants who consumed any types of dietary supplements with antioxidant properties (such as vitamin C, vitamin E and curcumin) and were allergic to astaxanthin, soya bean, or soybean oil were excluded from the study. The participants were also excluded if they smoke cigarette, drink alcohol and experience chronic diseases such as diabetes, dyslipidemia, liver or kidney disease as well as use immunosuppressive agents. Pregnant and breast-feeding mother were not allowed to participate in this study. Moreover, individuals who had participated in other clinical trials, or finished the trials for less than 6 months were not eligible.

This present study was approved by The Ethics Review Committee for Research Involving Human Research Subjects, Health Science Group, Chulalongkorn University. All of participants were acknowledged and signed informed consent before participating the study.

3.2 Sample size calculation

According to Chamnijarakit, (2001), to compare the effects of before and after treatments, the following formula was applied to estimate the sample size:

$$n = \left(\frac{(Z\alpha + Z\beta)(SD)}{D} \right)^2$$

Yoshida et al., (2010) suggested 6 mg of astaxanthin supplementation for 12 weeks could increase HDL-C levels. The difference of HDL-C before and after supplementation was 10.6 (D), and the standard deviation was 10.8 (SD). The level of significance (α) was set at 0.05, "$Z\alpha$" = 1.96. The power of test "(β)" was set at 0.10, "$Z\beta$" = 1.28. The sample size was calculated as below:

$$n = \left(\frac{(1.96 + 1.28)(10.8)}{10.6} \right)^2$$

$$n = 10$$

To solve dropping out problem, the number (n) was adjusted for 40%

$$N = \frac{n}{(1-0.40)}$$

$$N = \frac{10}{(1-0.40)}$$

$$N = 17$$

Therefore, the requisite number of participants in each group was 17, and the total requirement of all participants in this present study was 34.

3.3 Study design

This study was a 12-week, randomized, placebo-controlled trial. The research protocol was briefly summarized as presented in **Figure 4**. A week before starting the experiment, all participants underwent physical examination and health screening by the physician to ensure whether they were healthy and met the inclusion criteria. Additionally, they also received 3-day food record form with the instruction of how to complete the form correctly. The completed 3-day food records were then returned to the researchers on the consecutive week.

At baseline, anthropometric measurement and blood pressure were recorded. Blood sampling were collected after an overnight fasting period for 8-12 hours. Then, the participants were allocated into either experimental or placebo groups by systematic randomization methods. The participants in the experimental group were supplemented with 4 mg of astaxanthin once daily after breakfast while those in the placebo group were supplemented with 147 mg of soybean oil once daily for 12 weeks. The astaxanthin and placebo capsules were identical in appearances. The participants also received 3-day food record forms to record their food intake during the last week of the study. Throughout the study period, the participants were encouraged to maintain their usual lifestyles, physical activities, and diets. At the end of the study (week 12), the participants underwent anthropometric measurement, blood pressure record and blood collection the same as at baseline. The completed 3-day food record form and the bottle containing astaxanthin and placebo capsules were also returned to the researchers.

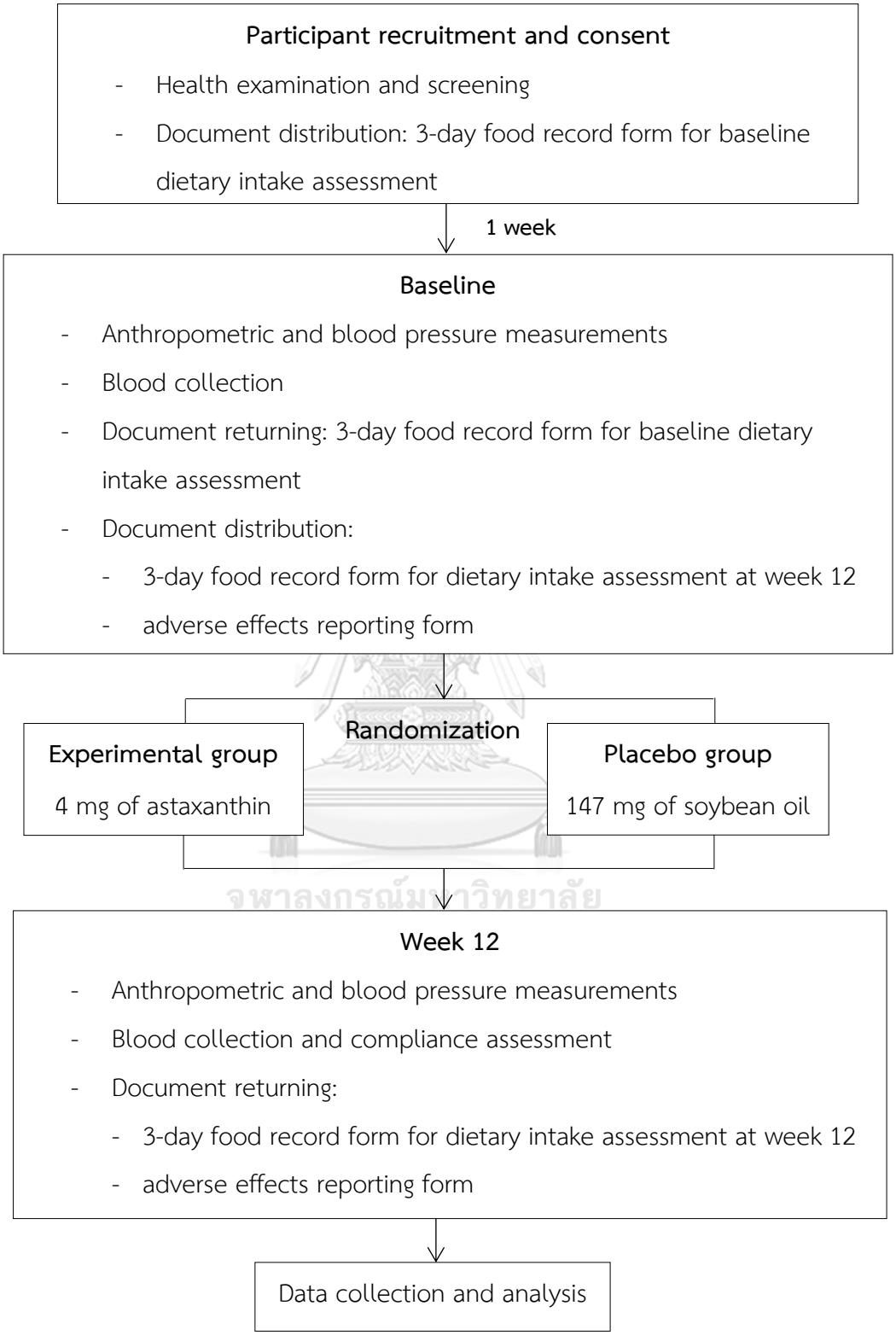

Figure 4 Diagram of research protocol

3.4 Astaxanthin supplement

Astaxanthin capsule was manufactured by MEGA LIFESCIENCES Public Co., Ltd., Thailand. The capsule consisted of 80 mg natural astaxanthin complex which provided astaxanthin for 4 mg as the active ingredient. The soybean oil 70 mg was used as inactive ingredient. The capsule shell made of gelatin, glycerin, and purified water. The placebo capsule consisted of 147 mg of soybean oil as inactive ingredient. The capsules of astaxanthin and placebo were identical in appearances including size, shape, and color.

3.5 Dietary intake assessment

Dietary intakes of the participants were assessed by 3-day food record at baseline and week 12 of the study. The 3-day food record consisted of meals, ingredients, quantities, cooking methods, and dining places. The participants had to record their food intake for 3 days (2 weekdays and 1 weekend day). The data from the dietary record were analyzed by INMUCAL-Nutrients software (V.3.0, Institute of Nutrition, Mahidol University, Thailand) (Institute of Nutrition, 2015).

3.6 Laboratory assessment

Blood collection were performed at baseline and week 12. The total of 20 milliliter venous blood samples were collected after an overnight fasting period for 8-12 hours to determine lipid profile (TC, HDL-C, LDL-C, and TG), fasting blood glucose (FBG), and complete blood count (CBC). Blood samples were analyzed at the Faculty of Allied Health Sciences, Chulalongkorn University, and serum astaxanthin were analyzed according to the method developed by Lazzarino et al., (2017).

3.7 Astaxanthin analysis

3.7.1 Preparation of astaxanthin stock solution

One milligram of all-trans-astaxanthin analytical standard (lot number BCBZ9727) was purchased from Sigma-Aldrich, MO, USA. The indirect weighing method was used to determine the amount of astaxanthin dry powder. First of all, the vial contained astaxanthin dry powder was weighted. Then, gradually dissolved astaxanthin powders by a little amount of HPLC-grade chloroform (Batch no. 16120096, RCI Labscan, Thailand) followed by transferring the solution to 50 milliliter-volumetric flask. This step was repeated until the solution in the vial was colorless. Then, adjusted the final volume to 50 milliliters by HPLC-grade chloroform. The next step was to leave the empty vial in desiccator until it was dried. The dried vial was then weighed. The latter weight was subtracted from the initial weight of the vial. Hence, the missing weight was the weight of astaxanthin dry powder which was then used to calculate the concentration of astaxanthin stock solution.

3.7.2 Preparation of astaxanthin standard solution and calibration curve

According to a single step preparation of biological fluids for HPLC analysis of antioxidants (Lazzarino et al., 2017), astaxanthin stock solution was diluted by HPLC-grade acetonitrile (Batch no. 17100226, RCI Labscan, Thailand) to 0.10, 0.30, 0.50, 1.00, and 2.00 µmol/L. Each 750 µL of the standard solutions were transferred into microcentrifuge tube and mixed by vortex for 1 minute. All of microcentrifuge tubes were incubated at 37 °C for 1 hour under agitation followed by centrifuging at 20,690 g for 15 minutes at 4 °C using Sorvall™ Legend™ Micro 21R, Thermo Fisher Scientific. Then, the standard solutions were filtered by 0.45 µm pore size microfiltration and preserved in ambler glass vial to further analysis by reversed-phase high performance liquid chromatography (RP-HPLC) (ProStar 410 Autosampler, Varian) using Hypersil Gold RP C18 column (100x4.6 nm, 5 µm particle size, Thermo Fisher Scientific) at wavelength of 479 nm. To generate calibration curve, area under the curve obtained

from RP-HPLC were plotted against concentration of standard solution. The regression equation obtained from this step was used to estimate serum astaxanthin concentration.

3.7.3 Determination of serum astaxanthin concentration

Once venous blood sample was collected, the sample was left for 30 minutes at room temperature and centrifuged at 10 °C, 1890g for 10 minutes using Eppendorf® Centrifuge 5430 R. Then, 250 µL of serum was collected and mixed with 500 µL acetonitrile followed by a single step preparation of biological fluids for HPLC analysis as described in 3.5.2 Astaxanthin concentration was then calculated according to calibration curve.

3.8 Evaluation of adverse effects and compliance

All participants were encouraged to observe adverse effects every day after taking the given capsules for 12 weeks. Any adverse effects found were reported in adverse effects reporting form. To evaluate participants' compliance, a weekly follow-up by telephone was performed to investigate both adverse effects and compliance. At the end of the study, the adverse effects reporting form was collected together with 3-day food record form from each participant. The remaining capsules were counted and calculated for percentage of compliance according to the following equation:

$$\text{Compliance (\%)} = \frac{\text{Consumed capsules}}{\text{Given capsules}} \times 100$$

The compliance of more than 80% is considered as good compliance.

3.9 Data collection and statistical analysis

The results were presented as a mean ± standard deviation (SD). Shapiro–Wilk test was used to analyze data normality. In case of normally distributed data, independent-samples t-test was used to compare means between two independent groups, and paired-sample t-test was used to compare means within group. When data were not normal distributed, non-parametric statistics were used. A value of $p < 0.05$ was defined as statistical significance. The statistical analysis in this study was performed by SPSS version 22 (SPSS Inc., Chicago, IL, 2013).

CHAPTER IV

RESULTS

This study aimed to investigate the effects of a 12-week astaxanthin supplementation on FBG, lipid parameters and hematological parameters in healthy volunteers. Compliance and any adverse effects of supplementation were also examined.

4.1 Baseline characteristics of participants

A total of 38 healthy participants (10 men and 28 women) were enrolled in this study. They were systematically randomized into the experimental group (n = 19) and the placebo group (n = 19). During study period, a total of 5 participants dropped out (3 in the experimental group and 2 in the placebo group) because they were unable to follow research protocol, and the reason for drop out was not related to the given intervention. Therefore, 33 participants (16 in the experimental group and 17 in the placebo group) were finally analyzed for dietary intake and serum astaxanthin concentration. However, one participant in the placebo group could not complete blood collection process. Thus, the biological parameters including FBG, lipid parameters and CBC in 16 participants of the placebo group were analyzed.

Baseline characteristics of the participants are presented in **Table 2**. There were 9 men and 24 women in this present study. Their average age were 33.39 ± 10.07 years (28.11 ± 5.47 years in men and 35.38 ± 10.76 years in women). The average weight and height of total participants were 56.70 ± 8.19 kg and 1.62 ± 0.08 m, respectively. The average BMI was 21.55 ± 2.08 kg/m^2. The average blood pressure including systolic and diastolic blood pressure were 111.14 ± 9.59 mmHg and 73.14 ± 7.90 mmHg, respectively. There were 17 participants (5 men and 12 women) in the

placebo group. The average age of the participants in this group were 30.20 ± 6.01 years in men and 35.27 ± 10.65 years in women. The average weight and height were 56.41 ± 8.24 kg and 1.62 ± 0.08 m, respectively, and the average BMI was 21.33 ± 2.16 kg/m^2. The average systolic blood pressure was 112.06 ± 11.05 mmHg, and diastolic blood pressure was 73.82 ± 8.20 mmHg. In the experimental group, there were 16 participants (4 men and 12 women) with the average age of 25.50 ± 3.70 years in men and 34.33 ± 10.99 years in women. The average weight and height of the participants in this group were 57.00 ± 8.40 kg and 1.61 ± 0.07 m, respectively. The average systolic and diastolic blood pressure were 110.16 ± 7.98 mmHg and 72.41 ± 7.77 mmHg, respectively. No significant differences in baseline characteristics of participants between groups were observed, and the ratio between males and females were comparable in both groups.

4.2 Effect of astaxanthin supplementation on serum astaxanthin

The levels of serum astaxanthin are presented in **Figure 5**. At baseline, serum astaxanthin concentration of all healthy participants ranged from 0.0006 ± 0.0027 µmol/L to 0.0029 ± 0.0076 µmol/L, and no significant difference in serum astaxanthin between groups was observed. However, after a 12-week of the study, serum astaxanthin concentrations were significantly changed from baseline. The participants in the placebo group showed significantly increased serum astaxanthin concentration from 0.0006 ± 0.0027 µmol/L to 0.0046 ± 0.0050 µmol/L (p = 0.011), while serum astaxanthin concentrations of the participants in the experimental group were significantly increased from 0.0029 ± 0.0076 µmol/L to 0.0256 ± 0.0288 µmol/L (p = 0.001). When compared the levels of astaxanthin between groups at week 12, the results indicated that the serum astaxanthin concentration of the participants in the experimental group was significantly 5-fold higher than that of the participants in the placebo group (p = 0.001).

Table 2 Baseline characteristics of the participants

Parameters	Total (n = 33)	Placebo group (n = 17)	Experimental group (n = 16)
Gender (male/female)	9/24	5/12	4/12
Age (year)	33.39 ± 10.07	34.59 ± 9.98	32.13 ± 10.34
Men	28.11 ± 5.47	30.20 ± 6.01	25.50 ± 3.70
Women	35.38 ± 10.76	35.27 ± 10.65	34.33 ± 10.99
Weight (kg)	56.70 ± 8.19	56.41 ± 8.24	57.00 ± 8.40
Height (m)	1.62 ± 0.08	1.62 ± 0.08	1.61 ± 0.07
BMI (kg/m^2)	21.55 ± 2.08	21.33 ± 2.16	21.77 ± 2.03
Blood pressure (mmHg)			
Systolic	111.14 ± 9.59	112.06 ± 11.05	110.16 ± 7.98
Diastolic	73.14 ± 7.90	73.82 ± 8.20	72.41 ± 7.77

Data are presented as mean ± SD.

BMI = Body mass index

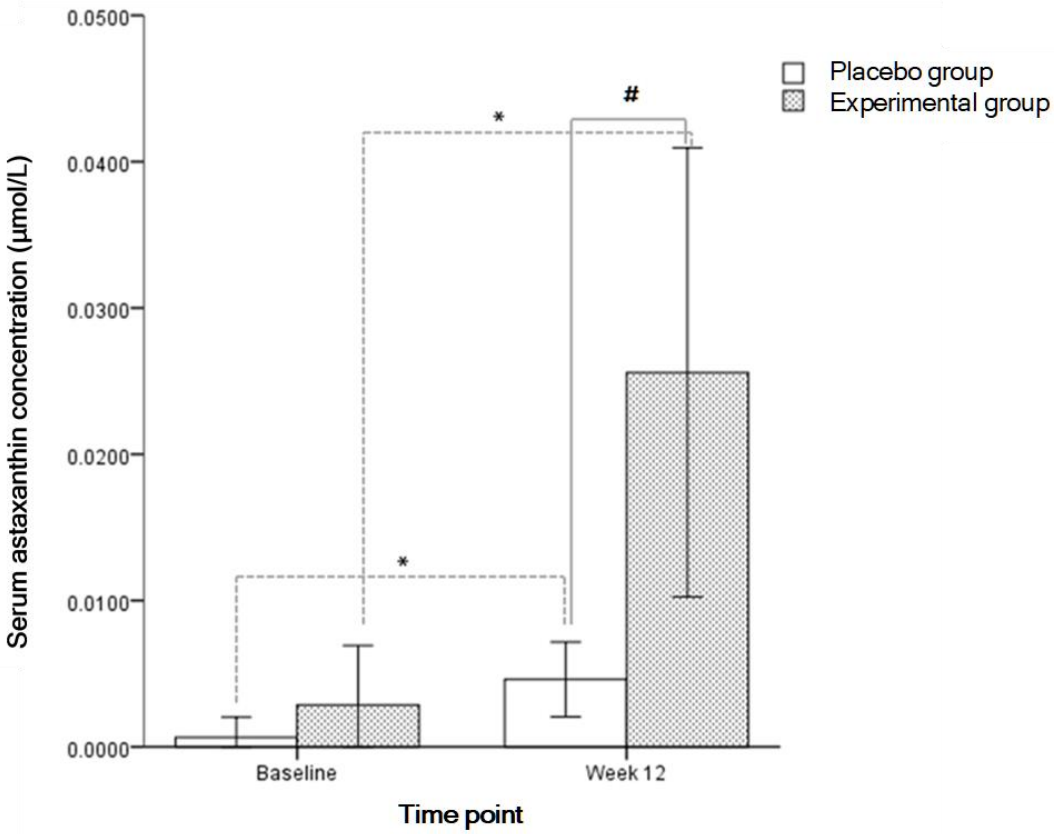

Figure 5 Serum astaxanthin concentrations of the participants

* Significant difference within group from baseline to week 12 ($p < 0.05$)

Significant difference between the groups at week 12 ($p < 0.05$)

4.3 Effects of astaxanthin supplementation on body weight, body mass index, and blood pressure

Table 3 shows body weight, body mass index (BMI) and blood pressure of the participants. The results showed no changes in body weight, BMI and blood pressure (both systolic and diastolic blood pressure) of the participants in both groups after 12-week supplementation. In addition, these parameters were not different between groups at week 12.

4.4 Effects of astaxanthin supplementation on FBG and lipid parameters

Table 4 shows the levels of FBG and lipid parameters of participants in the two groups. At baseline, FBG levels were 83.38 ± 6.36 mg/dL and 83.75 ± 6.17 mg/dL in the placebo group and in the experimental group, respectively. There was no difference in FBG level between groups at baseline. After 12 weeks of supplementation, FBG in both groups were still not significantly changed. At week 12, the levels of FBG were 84.56 ± 4.35 mg/dL and 83.94 ± 7.20 mg/dL in the placebo group and in the experimental group, respectively. No difference in FBG levels was observed between groups at this time point.

There were no significant differences in the levels of TC, HDL-C, and LDL-C between the groups at baseline; nevertheless, the difference in TG level was detected between groups. The participants in the experimental group showed significantly lower TG levels at baseline compared to those in the placebo group (p = 0.005). After 12-week of astaxanthin supplementation, the levels of TC, HDL-C and LDL-C of the participants in both groups did not change from baseline. However, at the end of the study, TG level of the participants in the experimental group was found to be significantly increased from 59.37 ± 19.36 mg/dL to 69.38 ± 21.13 mg/dL (p = 0.023). To describe more about the TG levels of the participants in the experimental group, the TG level of each participant in this group at baseline and week 12 was presented in **Figure 6**. The result showed that most of the participants (75%) in the experimental group had baseline TG less than 70 mg/dL. At the end of the study, no differences in any lipid parameters between groups were observed.

Table 3 Body weight, body mass index, and blood pressure of the participants

Parameters	Placebo group (n = 17)		Experimental group (n = 16)	
	Baseline	Week 12	Baseline	Week 12
Body weight (kg)	56.41 ± 8.24	56.44 ± 7.34	57.00 ± 8.40	57.41 ± 8.42
BMI (kg/m^2)	21.33 ± 2.16	21.30 ± 1.88	21.77 ± 2.03	21.87 ± 2.05
Blood pressure (mmHg)				
Systolic	112.06 ± 11.05	108.65 ± 9.60	110.16 ± 7.98	107.22 ± 9.98
Diastolic	73.82 ± 8.20	71.47 ± 10.57	72.41 ± 7.77	69.75 ± 4.61

Data are presented as mean ± SD.

BMI = body mass index

Table 4 The levels of blood glucose and lipid parameters of the participants

Parameters	Placebo group (n = 16)		Experimental group (n = 16)	
	Baseline	Week 12	Baseline	Week 12
FBG (mg/dL)	83.38 ± 6.36	84.56 ± 4.35	83.75 ± 6.17	83.94 ± 7.20
TC (mg/dL)	211.50 ± 34.54	217.63 ± 39.65	192.31 ± 27.66	198.13 ± 31.81
TG (mg/dL)	83.06 ± 24.16	85.81 ± 34.73	59.37 ± 19.36#	69.38 ± 21.13*
HDL-C (mg/dL)	61.25 ± 11.90	59.25 ± 10.80	59.63 ± 11.83	56.19 ± 10.95
LDL-C (mg/dL)	131.56 ± 34.22	141.13 ± 36.90	120.81 ± 23.78	128.44 ± 26.27

Data are presented as mean ± SD.

FBG = fasting blood glucose; TC = total cholesterol; TG = triglyceride; HDL-C = high-density lipoprotein cholesterol; LDL-C = low-density lipoprotein cholesterol

Significant difference between the groups at baseline ($p < 0.05$)

* Significant difference within group from baseline to week 12 ($p < 0.05$)

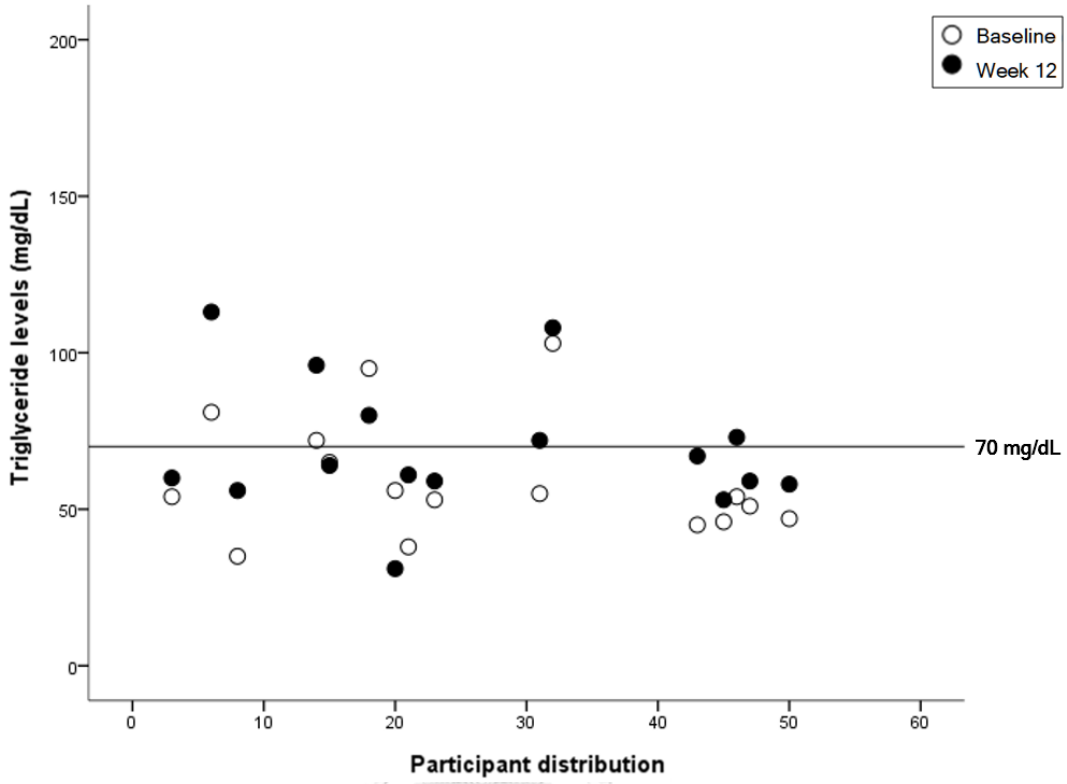

Figure 6 Triglyceride level of each participant in the experimental group at baseline and week 12

4.5 Effect of astaxanthin supplementation on hematological parameters

Hematological parameters were investigated at baseline and week 12 as presented in **Table 5**. At baseline, hematological parameters in the two groups were not different. After 12-week of supplementation, changes in MCV and MCHC were observed in both groups. MCV significantly increased from 83.78 ± 5.73 fL to 85.63 ± 6.24 fL in the placebo group (p = 0.001) and 84.86 ± 4.56 fL to 86.54 ± 4.95 fL in the experimental group (p = 0.001). MCHC significantly decreased from 33.07 ± 0.88 g/dL to 32.41 ± 1.10 g/dL in the placebo group (p = 0.001) and 33.05 ± 0.90 g/dL to 32.32 ± 1.06 g/dL in the experimental group (p = 0.001). In the placebo group, the results showed significant increase in platelet from 272.13 ± 54.44 (10^3/µL) to 294.13 ± 57.96 (10^3/µL) at week 12 (p = 0.004). Moreover, it was found that basophils significantly increased from 0.34 ± 0.21% to 0.46 ± 0.19% in the experimental group (p = 0.024). However, no significant differences in hematological parameters were found between the experimental and placebo groups at baseline and week 12.

4.6 Correlation between serum astaxanthin and biological parameters

To determine the relationship between FBG, lipid parameters, and serum astaxanthin, correlation between these parameters of all participants in both groups were performed at week 12. The results are presented in **Figure 7.** Serum astaxanthin correlate with FBG (p = 0.018). However, no correlation between serum astaxanthin, FBG, and lipid parameters were observed.

Table 5 Hematological parameters of the participants

Parameters	Placebo group (n= 16)		Experimental group (n= 16)	
	Baseline	Week 12	Baseline	Week 12
WBC (10^3/μL)	5.84 ± 1.13	6.09 ± 1.36	6.13 ± 1.30	6.16 ± 1.38
RBC (10^6/μL)	4.79 ± 0.52	4.81 ± 0.60	4.71 ± 0.42	4.73 ± 0.46
Hb (g/dL)	12.93 ± 1.57	12.89 ± 1.40	13.13 ± 1.16	13.09 ± 1.33
Hct (%)	39.07 ± 4.10	39.72 ± 3.70	39.69 ± 3.14	40.44 ± 3.46
MCV (fL)	83.78 ± 5.73	85.63 ± 6.24*	84.86 ± 4.56	86.54 ± 4.95*
MCH (pg)	27.54 ± 2.19	27.66 ± 2.06	28.05 ± 2.02	28.05 ± 2.01
MCHC (g/dL)	33.07 ± 0.88	32.41 ± 1.10*	33.05 ± 0.90	32.32 ± 1.06*
Platelet (10^3/μL)	272.13 ± 54.44	294.13 ± 57.96*	268.19 ± 70.88	281.44 ± 69.68
RDW (%)	14.04 ± 0.98	13.98 ± 1.01	13.67 ± 0.98	13.64 ± 1.17
Lymphocytes (%)	38.07 ± 8.18	36.57 ± 6.17	35.74 ± 8.29	35.20 ± 7.11
Monocytes (%)	5.84 ± 1.04	5.54 ± 1.07	6.08 ± 1.07	6.32 ± 1.66
Neutrophils (%)	52.84 ± 7.87	54.08 ± 6.57	54.65 ± 7.02	54.93 ± 7.42
Eosinophils (%)	3.06 ± 1.72	3.26 ± 2.26	2.88 ± 1.31	3.20 ± 1.86
Basophils (%)	0.36 ± 0.13	0.41 ± 0.17	0.34 ± 0.21	0.46 ± 0.19*

Data are presented as mean ± SD.

WBC = white blood cell; RBC = red blood cell; Hb = hemoglobin; Hct = Hematocrit; MCV = mean corpuscular volume; MCH = mean corpuscular hemoglobin; MCHC = mean corpuscular hemoglobin concentration; RDW = red cell distribution width

* Significant difference within group from baseline to week 12 ($p < 0.05$)

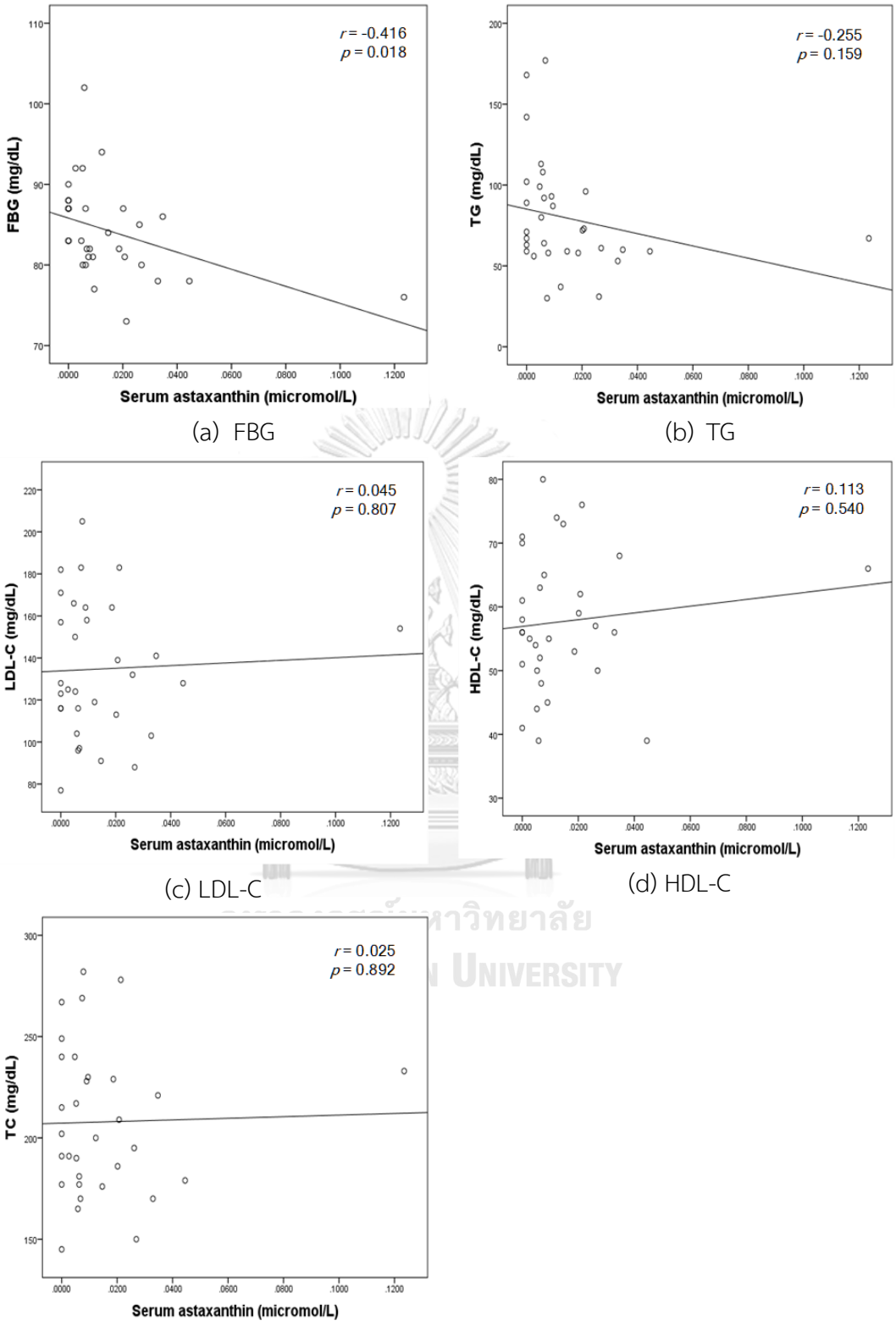

Figure 7 Correlation between serum astaxanthin and biological parameters at week 12
FBG = fasting blood glucose; TG = triglyceride; LDL-C = low-density lipoprotein cholesterol; HDL-C = high-density lipoprotein cholesterol; TC = total cholesterol

4.7 Effect of astaxanthin supplementation on dietary intake

Macronutrient intake was assessed as presented in **Table 6**. Baseline total energy intake of the participants in the placebo and the experimental groups were approximately 1,600 kcal/day and 1,780 kcal/day, respectively, and there was no significant difference between groups at baseline. However, at week 12, the participants in the experimental group showed significantly decreased total energy intake to approximately 1,500 kcal/day when compared to baseline (p = 0.010). In addition to total energy intake, it was found that carbohydrate intake of the participants in the experimental group significantly decreased approximately from 188 g/day to 155 g/day (p = 0.015). Therefore, the significantly decreased caloric intake from carbohydrate (752.22 ± 285.40 kcal to 620.38 ± 187.22 kcal) was observed in the experimental group (p = 0.015). Throughout the study, no changes in total energy and carbohydrate intakes were found in the placebo group. For fat intake, the participants in the placebo group and the experimental group consumed fat approximately 58.03 ± 24.75 g/day and 70.51 ± 25.74 g/day, respectively at baseline. At week 12, fat intake of the participants was 60.86 ± 21.16 g/day in the placebo group and was 62.88 ± 19.88 g/day in the experimental group. No differences in fat intake between groups were observed at baseline and at week 12. The ratio of energy distribution from carbohydrate, fat and protein of the participants was 50 : 32 : 18 in the placebo group and 45 : 34 : 20 in the experimental group. At week 12, the participants in the placebo group showed energy distribution of 49 : 33 : 19 while the energy distribution of those in the experimental group was 43 : 36 : 21.

For micronutrient intake **(Table 7),** baseline sodium consumptions of participants in the placebo and the experimental groups were approximately 2,500 mg/day and 2,700 mg/day, respectively. The difference in sodium consumption was observed at week 12. The participants in the placebo group consumed sodium approximately 2,800 mg/day whereas those in the experimental group consumed sodium approximately 2,100 mg/day. The results indicated that sodium consumption

of participants in the experimental group significantly lowered than those in the placebo group at week 12 (p = 0.014). In this study, there were no differences in other micronutrient intakes between the placebo and experimental groups after 12-week of 4 mg astaxanthin supplementation.

4.8 Compliance and adverse effects

In this study, the participants were randomly administered 4 mg of astaxanthin or placebo once daily for 12 consecutive weeks. According to the compliance evaluation, percentages of compliance were comparable in both groups (96.65 ± 11.11% in the experimental group and 96.64 ± 13.42% in the placebo groups). The results indicated that all participants had good compliance.

For adverse effect reporting outcomes, some undesirable effects were notified. Mild itching for a few days were reported (12.50% in the experimental group and 5.80% in the placebo group). According to participants' report, the itching was minor and became better without discontinuation of the given supplementation. In the experimental group, 5.80% of participants complained of having red stool, and 12.50% complained of losing appetite. Likewise, in the placebo group, 5.80% of the participants complained of nausea. However, their symptoms appeared during the first few weeks after intervention and disappeared without discontinuation of the supplement.

Table 6 Macronutrient intake of the participants

Parameters	Placebo group (n= 17)		Experimental group (n= 16)	
	Baseline	Week 12	Baseline	Week 12
Energy (kcal)	1,598.94 ± 438.21	1,619.30 ± 426.40	1,776.42 ± 503.85	1,519.04 ± 318.39*
CHO				
g	191.01 ± 57.78	188.98 ± 55.32	188.05 ± 71.35	155.09 ± 46.80*
kcal	764.02 ± 231.14	755.93 ± 221.28	752.22 ± 285.40	620.38 ± 187.22*
% of TE	50.19 ± 11.98	48.75 ± 8.34	45.45 ± 11.13	43.22 ± 8.24
Fat				
g	58.03 ± 24.75	60.86 ± 21.16	70.51 ± 25.74	62.88 ± 19.88
kcal	522.30 ± 222.72	547.70 ± 190.43	634.61 ± 231.68	565.92 ± 178.91
% of TE	31.72 ± 8.32	32.56 ± 5.72	34.03 ± 8.19	35.61 ± 6.31
Protein				
g	72.00 ± 31.40	75.05 ± 22.30	88.08 ± 38.37	83.13 ± 27.42
kcal	287.98 ± 125.63	300.20 ± 89.18	352.34 ± 153.48	332.50 ± 109.66
% of TE	18.08 ± 5.09	18.70 ± 4.60	20.52 ± 7.85	21.20 ± 5.53
Energy distribution				
CHO: Fat: Protein	50 : 32 : 18	49 : 33 : 19	45 : 34 : 20	43 : 36 : 21
Cholesterol (g)	296.67 ± 136.20	254.18 ± 134.60	307.90 ± 202.31	340.29 ± 150.61

Data are presented as mean ± SD.

CHO = carbohydrate; TE = total energy

* Significant difference within group from baseline to week 12 ($p < 0.05$)

Table 7 Micronutrient intake of the participants

Parameters	Placebo group (n= 17)		Experimental group (n= 16)	
	Baseline	Week 12	Baseline	Week 12
Sodium (mg)	2,532.14 ± 858.94	2,862.61 ± 846.84	2,683.70 ± 1,119.45	2,127.74 ± 759.11[#]
Vitamin C (mg)	39.81 ± 21.55	42.37 ± 30.05	32.26 ± 20.63	42.37 ± 30.05
Vitamin B1 (mg)	1.57 ± 1.56	1.48 ± 0.83	2.39 ± 2.03	2.11 ± 1.32
Vitamin B2 (mg)	1.58 ± 1.11	1.23 ± 0.56	1.70 ± 1.31	1.46 ± 0.67
Niacin (mg)	16.33 ± 7.85	16.41 ± 7.40	21.27 ± 11.16	17.63 ± 5.81
Vitamin B6 (mg)	0.76 ± 0.79	0.71 ± 0.63	1.41 ± 1.37	0.93 ± 0.85
Vitamin B12 (mg)	2.05 ± 0.97	1.85 ± 1.21	1.98 ± 1.73	1.85 ± 1.11
Retinol (µg)	458.85 ± 338.51	338.04 ± 211.89	272.00 ± 348.12	351.04 ± 213.98
Vitamin E (µg)	1.19 ± 0.87	1.48 ± 1.28	1.12 ± 0.91	1.15 ± 0.99
Beta-carotene (µg)	1,331.94 ± 980.49	920.31 ± 734.62	683.60 ± 829.18	849.83 ± 728.68

Data are presented as mean ± SD.

[#] Significant difference between the groups at week 12 ($p < 0.05$)

CHAPTER V

DISCUSSION

This present study investigated the effects of 12-week astaxanthin supplementation on general biological parameters including blood glucose, lipid parameters, and hematological parameters in healthy volunteers.

5.1 Effect of astaxanthin supplementation on serum astaxanthin concentration

This study highlighted the significantly increased serum astaxanthin concentration in the participants who were treated with 4 mg astaxanthin for 12 consecutive weeks. In the experimental group, astaxanthin concentration increased from 0.0029 µmol/L, which was nearly undetectable, to 0.0256 µmol/L after 12-week of astaxanthin supplementation. Astaxanthin, a powerful antioxidant of xanthophyll carotenoids, is commonly found in marine food sources such as salmon, shrimp, crab as well as algae (Higuera-Ciapara et al., 2006). *Haematococcus pluvialis* is the microalgae that considered as the highest source of natural astaxanthin available in commercial dietary supplements (Ambati et al., 2014; He et al., 2007). In facts, it is believed that astaxanthin cannot be synthesized in humans' body. For this reason, humans acquire astaxanthin mainly by consuming astaxanthin-rich food sources (Yang et al., 2013).

In the present study, the significant elevated astaxanthin concentration for approximately 9-fold from baseline after 12-week astaxanthin supplementation correspondingly represented the good compliance of the participants. The finding was consistent with the previous studies that oral administration of astaxanthin could raise the concentration of astaxanthin in blood. As stated by Karppi et al. (2007), plasma astaxanthin concentration in healthy men increased from almost undetectable to 0.0032 µmol/L when those participants were supplemented with 8

mg astaxanthin for 12 weeks. Park et al. (2010) found that plasma astaxanthin increased from undetectable to 0.14 μmol/L after supplementation with 8 mg of astaxanthin for 4 weeks which was considered as a short period. Miyazawa et al. (2014) presented the increased plasma astaxanthin from undetectable to 0.0182 μmol/L after administration of 1 mg/day of astaxanthin for 12 weeks. In addition, they found that 3 mg/day of astaxanthin for the same duration could raise plasma astaxanthin from nearly undetectable to 0.0624 μmol/L. The elevated blood astaxanthin concentration indicated that astaxanthin was well absorbed from gastrointestinal tract.

The absorption of astaxanthin is quite resemble to that of other lipids absorption (Ambati et al., 2014). It was occurred by passive diffusion into intestinal epithelium followed by the incorporation of carotenoids into chylomicrons before transportation to the liver and distribution to target tissues (Desmarchelier and Borel, 2017). At the beginning of the study, participants were informed to administer astaxanthin with meal or immediately after meal. This is because fat composition in foods can enhance astaxanthin absorption (Odeberg et al., 2003). Thus, administration of astaxanthin after meal is the crucial factor that may reflect the concentration of astaxanthin in blood. However, the increased level may vary depending on several factors including dosage of astaxanthin, intervention period, and baseline characteristics of each participant.

This study also found the increased serum astaxanthin concentration of the participants in the placebo group at week 12 compared to baseline. The concentration increased from nearly undetectable to 0.0046 μmol/L. This was actually still considered as a very low concentration. The explanation of this finding may be related to the participant's food consumption. Since the participants were encouraged to maintain their lifestyle behaviors, they freely consumed a variety of foods as they usually did. The results of 3-day food record indicated that some participants frequently consumed astaxanthin-containing foods such as egg yolk,

shrimp, and salmon. Therefore, it might be possible that certain amount of astaxanthin in their blood was detected. The previous evidence suggested that regularly consumption of salmon could lead to the absorption of astaxanthin into plasma (Rüfer et al., 2008).

5.2 Effect of astaxanthin supplementation on FBG

This present study investigated the effects of 4 mg astaxanthin supplementation for 12 weeks on FBG in healthy individuals. Serum astaxanthin concentration tended to correlated with FBG, but the result was not significant. At week 12, there was no significant difference in FBG between groups. Although the previous study suggested the benefits of astaxanthin on blood glucose metabolism of type 2 diabetes patients, the supplementation by 4 mg of astaxanthin for 12 weeks seemed to have no impact on FBG of healthy participants (Mashhadi et al., 2018). The results of this present study were in agreement with those found by earlier study (Nakagawa et al., 2011), in which the FBG of healthy subjects did not change after 6 and 12 mg of astaxanthin supplementation for 12 weeks. Another study also found that 12 mg of astaxanthin supplementation for 4 weeks did not affect the FBG levels of healthy individuals (Saito et al., 2012). Hence, this can imply that astaxanthin supplementation may have less effect on FBG in healthy individuals with normal FBG levels.

In animal studies, astaxanthin supplementation was purposed to reduce FBG via several mechanisms. In type 2 diabetes mice, astaxanthin protected beta cells from glucose toxicity by reducing FBG and the hyperglycemia condition induced by oxidative stress as well as increasing insulin levels (Uchiyama et al., 2002). In mice treated with high fat and high fructose diet, astaxanthin supplementation promoted the IRS–PI3K–Akt pathway of insulin signaling by lowering serine phosphorylation and enhanced glucose metabolism by controlling metabolic enzymes (Bhuvaneswari and

Anuradha, 2012). Moreover, *in vitro* study suggested that astaxanthin can ameliorate insulin resistance by protecting cells from oxidative stress (Ishiki et al., 2013).

5.3 Effect of astaxanthin supplementation on lipid parameters

The results of this present study pointed out that 4 mg of astaxanthin supplementation did not affect the levels of TC, HDL-C, and LDL-C. The results were consistent with previous studies. Researchers found that astaxanthin supplementation at the dose of 8 mg for 12 weeks did not alter the levels of TC, HDL-C, and LDL-C in healthy men aged 19-33 years (Karppi et al., 2007). Another study conducted by Baralic et al., (2013) showed that 4 mg of astaxanthin supplementation for 12 weeks did not affect those lipid parameters of soccer players. Furthermore, Nakagawa et al. (2011) also found that 6 and 12 mg of astaxanthin supplementation for 12 weeks did not change TC, HDL-C, and LDL-C levels of middle-aged and senior participants. Conversely, a study found by Yoshida et al. (2010) suggested that astaxanthin supplementation at the dose of 6 and 12 mg for 12 weeks could increase HDL-C level.

Even though the mechanisms of astaxanthin in controlling dyslipidemia in humans are still insufficient, it is believed that adiponectin is the key factor correlate with the changing of HDL-C level. The *in vitro* study suggested that adiponectin might improve apolipoprotein A-1-mediated cholesterol efflux from macrophages via ATP-binding cassette transporter A1-dependent pathway by means of activating liver X receptor alpha and PPAR gamma, resulting in the increased HDL-cholesterol (Tsubakio-Yamamoto et al., 2008). However, another study found that astaxanthin did not regulate liver X receptor alpha and PPAR gamma but amplified the expression of ATP-binding cassette transporter A1/G1 and the efflux of cholesterol from macrophages (Iizuka et al., 2012). Currently, the knowledge associated with the effects of astaxanthin on lipid parameters in humans is unclear, and the effects may

fluctuate in healthy individuals depending on several factors including the dose, duration of study, gender, and age of participants.

In the present study, TG level of the participants in the experimental group was significantly increased after supplementation with 4 mg of astaxanthin. The result disagreed with the previous studies. Choi et al., (2011b) found no positive effects of high dose astaxanthin (20 mg/day) supplementation for 12 weeks on TG level in overweight participants. MacDermid et al., (2012) also found no effects of 4 mg astaxanthin for 12 weeks on TG level in the participants with carpal tunnel syndrome. In addition, the study by Saito et al., (2012) revealed that 12 mg/day of astaxanthin supplementation for 4 weeks did not affect TG level in healthy participants. Currently, no published data notified the increased TG level after astaxanthin supplementation. The previous study conducted by Baralic et al., (2013) found that TG level of the athletes tend to be increased from 0.84 ± 0.12 mmol/L at baseline to 0.91 ± 0.08 mmol/L at day 45. However, this was nonsignificant, and the levels seemed to return back to near normal at week 12.

When thoroughly examined the baseline TG level of the participants in the experimental group, it appeared that 10 out of 13 participants had baseline TG level less than 70 mg/dL. In healthy individuals, TG level less than 90 mg/dL is normally considered as an acceptable value (Grundy et al., 2018). However, when TG level is less than 70 mg/dL, it could be considered as hypotriglyceridemia (Boemeke et al., 2015). In some conditions, extremely low TG was considered as the factor associated with health complication; for example, malnutrition and thyroid disorders (Carr et al., 1989; Elsurer et al., 2008; Lee et al., 2019). Thus, if the antioxidant could raise TG to the normal level, it might be beneficial to those with health complications. However, further research is required to verify the effects of astaxanthin on TG level, especially in those who had TG lower than 70 mg/dL.

5.4 Effect of astaxanthin supplementation on hematological parameters

After supplementation with 4 mg of astaxanthin for 12 weeks, most of the hematological parameters were not altered. The findings related to no effects of astaxanthin on hematological parameters agreed with the previous studies. Spiller and Dewell (2003) suggested that 6 mg of astaxanthin per day did not alter any blood cell count at week 4 and week 8 in healthy adults aged 35-69 years. Nakagawa et al. (2011) showed no effects of 6 and 12 mg of astaxanthin for 12 weeks on hematological parameters of middle-aged participants. However, they found that astaxanthin could decrease erythrocyte phospholipid hydroperoxides concentrations, resulting in enhanced erythrocyte antioxidant status. Moreover, in open-label study conducted in participants with oxidative stress burden also indicated that 12 mg of astaxanthin supplementation for 8 weeks did not produce any undesirable effects on hematological parameters (Iwabayashi et al., 2009). Astaxanthin were found to reduce blood transit time which then improved blood rheology after supplemented with 6 mg of astaxanthin for 10 days in middle-aged adults (Miyawaki et al., 2008).

This present study found significant increased basophils of participants in the experimental group at the end of the study. However, the alteration was minor and the value of basophils was still in normal range (0.00-1.00%) regarding to the references range obtained from Faculty of Allied Health Sciences, Chulalongkorn University. Significant increased platelet (about 8% from baseline) of the participants in the placebo group at week 12 was also observed in this study, but such changed level was still within normal range. A small fluctuation of platelet in healthy adults may occur since platelet lifespan of humans ranges from 8-12 days (Josefsson et al., 2013). In addition to platelet alteration at week 12, the minor changes in MCV and MCHC of participants in both groups were observed, and the levels were still within normal range. In fact, MCV is the average size of red blood cell, and MCHC is the concentration of hemoglobin in red blood cell (Dean, 2005). When red blood cell is

senescent, the alteration of MCV and MCHC could be occurred. Red blood cells turnover is around 120 days (Arias and Arias, 2017).

5.5 Effect of astaxanthin supplementation on dietary intake

This present study revealed the effect of astaxanthin on dietary intake. Four milligrams of astaxanthin supplementation for 12 weeks could lead to decreased total energy and carbohydrate intakes in healthy volunteers. In addition, the participants supplemented with astaxanthin for 12 weeks had significant lower sodium intake than those treated with placebo. According to dietary reference intake (DRI) for Thais 2020 (Department of Health, 2020), baseline energy consumption of the participants in the experimental group met the general recommendation. After supplementation with astaxanthin, the total energy intake decreased to lower than the recommendation. This could be the result of astaxanthin supplementation that affected food consumption. Thus, the result suggested the potential dietary intake lowering effect of astaxanthin in these individuals. Further studies are required to clarify this outcome. As the majority of total caloric intake come from carbohydrate, decreased carbohydrate consumption certainly affected the total caloric intake per day (Austin et al., 2011). The result of the present study agreed with this previous study as the decreased total energy intake was found along with decreased carbohydrate intake after astaxanthin supplementation. For other macronutrient consumption, the participants in the experimental group consumed sufficient amount of fat and protein based on the recommendation. Following astaxanthin supplementation, no alteration in these macronutrients were observed.

Other than astaxanthin, the inactive ingredient mainly consisted in the capsule was soybean oil. The soybean oil showed several benefits in pharmaceutical dosage form (Karasulu et al., 2011). For example, serving as a solvent to dissolve astaxanthin, acting as a barrier to oxygen to decrease oxidation, and assisting in a pigment barrier (Chen and Meyers, 1982). In this present study, soybean oil was used

as capsule base in small amount. The soybean oil provide less energy which was not affect the interested outcomes. Only high dose of soybean oil consumption affect biological parameters. The total of 15.7 g soybean oil per day consisted of 4.2 g of stearidonic acid showed positive effects on cardiovascular disease protection (Lemke et al., 2010). Moreover, about 20 g of soybean oil per day seemed to have benefits in lowering cardiac events (Harris et al., 2008).

For micronutrient intake, sodium is the main micronutrient found in several food sources (Satheannoppakao et al., 2013). Reducing food intake usually reduces sodium intake. According to Thai DRI 2020 (Department of Health, 2020), sodium intake should not more than 1,450 mg/day in male and 1,200 mg/day in female aged 19-60 years. Dietary intake assessment of the participants in this present study pointed out the excess sodium consumption than the recommendation. Astaxanthin supplementation lowered sodium intake in the experimental group possibly due to reduced food consumption. Owing to the dietary intake-lowering effect of astaxanthin, it might be beneficial in limiting sodium intake. For other micronutrient intake, in general, participants in both groups consumed sufficient amount of micronutrients. This could be the result of sufficient consumption of micronutrient food sources.

The results of this present study were inconsistent with those in previous studies. Mashhadi et al. (2018) performed dietary intake assessment using 3-day food records to estimate daily nutrients intake in type 2 diabetes patients who supplemented with 8 mg astaxanthin for 8 weeks. They revealed no effects of astaxanthin on any nutrient intake. Another study by Karppi et al. (2007) evaluated the effects of 8 mg astaxanthin supplementation for 12 weeks on lipid peroxidation, and 4-day data collection was used to assess nutrient intake of healthy participants. Although energy intake seemed a bit lower at week 12 compared to baseline, but no significant change was found (Karppi et al., 2007). Similarly, Iwamoto et al. (2000) showed no differences in dietary nutrient intakes, assessed by 3-day food record

before and during experimental period, of participants administered 1.8, 3.6, 14.4, and 21.6 mg of astaxanthin for 14 days.

According to the research area of antioxidants and food consumption, several works reported the positive effects of specific antioxidants on dietary intake in order to control body weight. For example, lipoic acid, a cofactor for pyruvate dehydrogenase that involved in energy metabolism process, could lead to body weight loss in obese subjects after 1,800 mg/day of alpha-lipoic supplementation for 20 weeks. The underlying mechanisms might include insulin sensitivity improvement and 5'-AMP-activated protein kinase activation of alpha-lipoic acid (Koh et al., 2011). Lycopene, a member of carotenoids, was found to control body weight in adult female rats by decrease serum ghrelin, the hormone regulating appetite stimulation. Therefore, body weight were decreased (Chronaiou et al., 2012; Jahromi et al., 2017).

Another carotenoids that was found to use as weight control agent was fucoxanthin (Maeda et al., 2007). It was believed that fucoxanthin could improve insulin sensitivity and increase energy expenditure (Gammone and D'Orazio, 2015). For astaxanthin, the previous study was conducted in moderate dyslipidemia with metabolic syndrome patients. The results revealed lower level of leptin as well as the improvement of leptin-to-adiponectin ratio after supplementation with dietary supplement containing astaxanthin (Ruscica et al., 2014). In fact, leptin made by white adipose tissues plays an important role in regulation of food intake and energy expenditure. In healthy individuals, secretion of leptin in fasting state leads to feeling hungry and increase food intake, and then leptin will return to normal level after eating (Kelesidis et al., 2010). An imbalance of leptin in some groups of people certainly affects food intake. For example, overweight patients who had leptin deficiency at newborn showed intense hyperphagia and impaired satiety (Yupanqui-Lozno et al., 2019). It has been found that increasing leptin levels of this patients can contribute to decreased food intake (Farooqi et al., 2002). Although the mechanism of the xanthophyll carotenoids such as astaxanthin in reducing food intake in

humans is still unclear, it is possible to explain by both neurohormornal pathways and metabolic alteration. Losing appetite which was one of the adverse events reported by some participants who were supplemented with astaxanthin may contribute to reduced caloric and carbohydrate intakes in the present study. Although the results showed decreased caloric and carbohydrate consumption, the participants' weight did not change at the end of the study. Further study is warranted to clarify the effect of astaxanthin on food consumption.

5.6 Adverse effects of astaxanthin supplementation

This present study reported no serious adverse effects of astaxanthin supplementation. Although mild itching was existed at the beginning of the intervention, the symptom was minor and disappeared without discontinuation of astaxanthin supplementation. This agreed with the safety study performed in healthy volunteers (Odeberg et al., 2003). In addition, a red-stained stool that was occasionally presented after astaxanthin supplementation in this study was in agreement with the adverse events found in previous studies (Kajita et al., 2009; Okada et al., 2009; Satoh et al., 2009). Losing appetite was reported in the participants supplemented with astaxanthin in the present study. This event has never been mentioned in any previous studies.

CHAPTER VI

CONCLUSION

This present study was implemented to investigate the effects of astaxanthin supplementation at the dose of 4 mg/d for 12 weeks on biological parameters in healthy adults. Serum astaxanthin concentration was significantly increased after astaxanthin supplementation. Although increased serum astaxanthin was explicitly found, there were no significant effects of astaxanthin on FBG, TC, LDL-C, HDL-C, and most of hematological parameters. In this study, total energy and carbohydrate intakes were significantly decreased from baseline after 12-week astaxanthin supplementation. Interestingly, the participants supplemented with astaxanthin also had lowered sodium intake than those supplemented with placebo. No serious adverse effects of astaxanthin supplementation were observed during study period. The mild itching, red stool and losing appetite were reported. These adverse events generally became better without discontinuing supplementation. The information obtained in this present study suggested no effects on general biological parameters and potential dietary intake-lowering effect of astaxanthin in healthy individuals.

Recommendation for further research

The future research may be conducted with larger sample size in order to increase the precision of the interested outcomes. Restriction of food containing astaxanthin may be considered to reduce confounding factor which can lead to misinterpretation of serum astaxanthin concentration in both experimental and placebo groups. Moreover, serum ghrelin, leptin and adiponectin may be investigated to clarify the effect of astaxanthin on dietary intake in healthy individuals.

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APPENDICES

APPENDIX A

Certificate of approval

AF 02-12

The Research Ethics Review Committee for Research Involving Human Research Participants, Health Sciences Group, Chulalongkorn University
Jamjuree 1 Building, 2nd Floor, Phyathai Rd., Patumwan district, Bangkok 10330, Thailand,
Tel/Fax: 0-2218-3202 E-mail: eccu@chula.ac.th

COA No. 179/2018

Certificate of Approval

Study Title No. 145.1/61 : EFFECTS OF ASTAXANTHIN SUPPLEMENTATION ON OXIDATIVE STRESS AND HEALTH IN HEALTHY INDIVIDUALS

Principal Investigator : ASSOC.PROF. KULWARA MEKSAWAN, Ph.D.

Place of Proposed Study/Institution : Faculty of Pharmaceutical Sciences, Chulalongkorn University

The Research Ethics Review Committee for Research Involving Human Research Participants, Health Sciences Group, Chulalongkorn University, Thailand, has approved constituted in accordance with the International Conference on Harmonization – Good Clinical Practice (ICH-GCP).

Signature: ..... Signature: .....

(Associate Professor Prida Tasanapradit, M.D.)
Chairman

(Assistant Professor Nuntaree Chaichanawongsaroj, Ph.D.)
Secretary

Date of Approval : 31 July 2018 Approval Expire date : 30 July 2019

The approval documents including

1) Research proposal
2) Patient/Participant Information Sheet and Informed Consent Form
3) Researcher
4) Questionnaire
5) Advertising leaflet

Protocol No. 145.1/61
Date of Approval 31 JUL 2018
Approval Expire Date 30 JUL 2019

The approved investigator must comply with the following conditions:

1. *The research/project activities must end on the approval expired date of the Research Ethics Review Committee for Research Involving Human Research Participants, Health Sciences Group, Chulalongkorn University (RECCU). In case the research/project is unable to complete within that date, the project extension can be applied one month prior to the RECCU approval expired date.*
2. *Strictly conduct the research/project activities as written in the proposal.*
3. *Using only the documents that bearing the RECCU's seal of approval with the subjects/volunteers (including subject information sheet, consent form, invitation letter for project/research participation (if available).*
4. *Report to the RECCU for any serious adverse events within 5 working days*
5. *Report to the RECCU for any change of the research/project activities prior to conduct the activities.*
6. *Final report (AF 03-12) and abstract is required for a one year (or less) research/project and report within 30 days after the completion of the research/project. For thesis, abstract is required and report within 30 days after the completion of the research/project.*
7. *Annual progress report is needed for a two- year (or more) research/project and submit the progress report before the expire date of certificate. After the completion of the research/project processes as No. 6.*

AF 02-12

The Research Ethics Review Committee for Research Involving Human Research Participants, Group I, Chulalongkorn University
Jamjuree 1 Building, 2nd Floor, Phyathai Rd., Patumwan district, Bangkok 10330, Thailand,
Tel: 0-2218-3202, 0-2218-3049 E-mail: eccu@chula.ac.th

COA No. 192/2019

Certificate of Approval

Study Title No. 145.1/61 (1) : EFFECTS OF ASTAXANTHIN SUPPLEMENTATION ON OXIDATIVE STRESS AND HEALTH IN HEALTHY INDIVIDUALS

Principal Investigator : ASSOC.PROF. KULWARA MEKSAWAN, Ph.D.

Place of Proposed Study/Institution : Faculty of Pharmaceutical Sciences, Chulalongkorn University

The Research Ethics Review Committee for Research Involving Human Research Participants, Health Sciences Group, Chulalongkorn University, Thailand, has approved constituted in accordance with Belmont Report 1979, Declaration of Helsinki 2013, Council for International Organizations of Medical Sciences (CIOM) 2016, Standards of Research Ethics Committee (SREC) 2013, and National Policy and guidelines for Human Research 2015.

Signature: Prida Tasanapradit
(Associate Prof. Prida Tasanapradit, M.D.)
Chairman

Signature: Nuntaree Chaichanawongsaroj
(Assistant Prof. Nuntaree Chaichanawongsaroj, Ph.D.)
Secretary

Date of Approval : 1 August 2019

Approval Expire date : 31 July 2020

The approval documents including;

1) Research proposal
2) Participant Information Sheet and Consent Form
3) Researcher
4) Questionnaire
5) Advertising leaflet

Approved No. 145.1/61
Date of Approval - 1 AUG 2019
Approval Expire Date 31 JUL 2020

The approved investigator must comply with the following conditions:

1. *The research/project activities must end on the approval expired date of the Research Ethics Review Committee for Research Involving Human Research Participants, Health Sciences Group, Chulalongkorn University (RECCU). In case the research/project is unable to complete within that date, the project extension can be applied one month prior to the RECCU approval expired date.*
2. *Strictly conduct the research/project activities as written in the proposal.*
3. *Using only the documents that bearing the RECCU's seal of approval with the subjects/volunteers (including subject information sheet, consent form, invitation letter for project/research participation (if available).*
4. *Report to the RECCU for any serious adverse events within 5 working days*
5. *Report to the RECCU for any change of the research/project activities prior to conduct the activities.*
6. *Final report (AF 02-14) and abstract is required for a one year (or less) research/project and report within 30 days after the completion of the research/project. For thesis, abstract is required and report within 30 days after the completion of the research/project.*
7. *Annual progress report is needed for a two- year (or more) research/project and submit the progress report before the expire date of certificate. After the completion of the research/project processes as No. 6.*

APPENDIX B

Informed consent

AF05-07

หนังสือแสดงความยินยอมเข้าร่วมการวิจัย

ทำที่.....

วันที่.....เดือน.....พ.ศ. .....

เลขที่ ประชากรตัวอย่างหรือผู้มีส่วนร่วมในการวิจัย.....

ข้าพเจ้า ซึ่งได้ลงนามท้ายหนังสือนี้ ขอแสดงความยินยอมเข้าร่วมโครงการวิจัย

ชื่อโครงการวิจัย ผลของการเสริมแอสตาแซนธินต่อภาวะเครียดออกซิเดชันและการอักเสบในผู้ที่มีสุขภาพดี

ชื่อผู้วิจัย รองศาสตราจารย์ เภสัชกรหญิง ดร.กุลวรา เมฆสวรรค์

ที่อยู่ที่ติดต่อ ภาควิชาอาหารและเภสัชเคมี คณะเภสัชศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย โทรศัพท์ 02-2188295, 0897796084

ข้าพเจ้า ได้รับทราบรายละเอียดเกี่ยวกับที่มาและวัตถุประสงค์ในการทำวิจัย รายละเอียดขั้นตอนต่างๆ ที่จะต้องปฏิบัติหรือได้รับการปฏิบัติ ความเสี่ยง/อันตราย และประโยชน์ซึ่งจะเกิดขึ้นจากการวิจัยเรื่องนี้ โดยได้อ่านรายละเอียดในเอกสารชี้แจงผู้เข้าร่วมการวิจัยโดยตลอด และได้รับคำอธิบายจากผู้วิจัย **จนเข้าใจเป็นอย่างดีแล้ว**

ข้าพเจ้าจึง**สมัครใจ**เข้าร่วมในโครงการวิจัยนี้ ตามที่ระบุไว้ในเอกสารชี้แจงผู้เข้าร่วมการวิจัย โดยข้าพเจ้ายินยอมรับประทานยาเม็ดแคปซูลซึ่งประกอบด้วยแอสตาแซนธิน 4 มิลลิกรัม หรือยาหลอก (placebo) อย่างใดอย่างหนึ่งด้วยวิธีการสุ่ม โดยรับประทานครั้งละ 1 แคปซูล วันละ 1 ครั้ง หลังอาหารเช้า เป็นเวลา 12 สัปดาห์ และมาพบผู้วิจัยก่อนและหลังการรับประทานผลิตภัณฑ์ดังกล่าว เพื่อรับการสัมภาษณ์เพื่อบันทึกข้อมูลในแบบบันทึกและแบบสอบถามต่างๆ ที่ใช้ในงานวิจัย ตลอดจนรับการชั่งน้ำหนัก วัดส่วนสูง และการเจาะเลือดจากเส้นเลือดดำประมาณครั้งละ 4 ช้อนชา *ทั้งหมด 2 ครั้ง ห่างกัน 12 สัปดาห์ ซึ่งระยะเวลาที่ใช้ในการมาร่วมการวิจัยแต่ละครั้งประมาณ 45 นาที เมื่อเสร็จสิ้นการวิจัยแล้วตัวอย่างเลือดที่เหลือหลังการวิเคราะห์ต่างๆ ข้างต้นของข้าพเจ้าจะถูกทำลาย*

ข้าพเจ้ามีสิทธิ**ถอนตัว**ออกจากการวิจัยเมื่อใดก็ได้ตามความประสงค์ **โดยไม่ต้องแจ้งเหตุผล** ซึ่งการถอนตัวออกจากการวิจัยนั้น จะไม่มีผลกระทบในทางใดๆ ต่อข้าพเจ้าทั้งสิ้น รวมถึงไม่มีผลกระทบต่อสิทธิการดูแลรักษาสุขภาพอื่นๆ ของข้าพเจ้า

ข้าพเจ้าได้รับคำรับรองว่า ผู้วิจัยจะปฏิบัติต่อข้าพเจ้าตามข้อมูลที่ระบุไว้ในเอกสารชี้แจงผู้เข้าร่วมการวิจัย และข้อมูลใดๆ ที่เกี่ยวข้องกับข้าพเจ้า ผู้วิจัยจะเก็บรักษาเป็น**ความลับ** โดยจะนำเสนอข้อมูลการวิจัยเป็นภาพรวมเท่านั้น ไม่มีข้อมูลใดในการรายงานที่จะนำไปสู่การระบุตัวข้าพเจ้า

หากข้าพเจ้าไม่ได้รับการปฏิบัติตรงตามที่ได้ระบุไว้ในเอกสารชี้แจงผู้เข้าร่วมการวิจัย ข้าพเจ้าสามารถร้องเรียนได้ที่คณะกรรมการพิจารณาจริยธรรมการวิจัยในคน กลุ่มสหสถาบัน ชุดที่ 1 จุฬาลงกรณ์มหาวิทยาลัย 254 อาคารจามจุรี 1 ชั้น 2 ถนนพญาไท เขตปทุมวัน กรุงเทพฯ 10330 โทรศัพท์/โทรสาร 0-2218-3202

E-mail: eccu@chula.ac.th

ข้าพเจ้าได้ลงลายมือชื่อไว้เป็นสำคัญต่อหน้าพยาน ทั้งนี้ข้าพเจ้าได้รับสำเนาเอกสารชี้แจงผู้เข้าร่วมการวิจัย และสำเนาหนังสือแสดงความยินยอมไว้แล้ว

ลงชื่อ.....
(.....)
ผู้วิจัยหลัก

ลงชื่อ.....
(.....)
ผู้มีส่วนร่วมในการวิจัย

ลงชื่อ.....
(.....)
พยาน

เลขที่โครงการวิจัย 145.1/61
วันที่รับรอง 31 ก.ค. 2561
วันหมดอายุ 30 ก.ค. 2562

1/1

V.2.4/2558

APPENDIX C

Documents for participants

(ส่วนที่ 5) **วิธีบันทึกการบริโภคอาหาร 3 วัน**

วันที่ควรจดบันทึกการรับประทานอาหาร ประกอบด้วย
- วันธรรมดา 2 วัน (วันจันทร์ ถึง วันศุกร์)
- วันหยุด 1 วัน (วันเสาร์ หรือ วันอาทิตย์)

↓

บันทึกการรับประทานอาหารและการดื่มเครื่องดื่มทุกมื้อ นับตั้งแต่มื้อเช้าจนถึงก่อนนอน
ทำการบันทึกขณะหรือหลังรับประทานอาหาร

คำชี้แจง ผู้วิจัยขอความร่วมมือในการบันทึกข้อมูลตามความเป็นจริง

ข้อมูลที่สำคัญของการจดบันทึกการบริโภคอาหาร 3 วัน การบันทึกในแต่ละวันประกอบด้วย

1. **ชนิดของมื้ออาหาร:** บันทึกมื้ออาหารที่รับประทาน (เช้า กลางวัน เย็น หรืออาหารว่าง) พร้อมทั้งระบุเวลาที่รับประทานโดยประมาณ
2. **รายการอาหารและเครื่องดื่ม:** บันทึกรายการอาหาร รวมทั้งเครื่องดื่มทุกชนิดที่รับประทาน เริ่มตั้งแต่ตื่นนอนจนกระทั่งก่อนเข้านอน เช่น ข้าวผัดกะเพราหมูไข่ดาว ช็อกโกแลตเย็น เป็นต้น
3. **ส่วนประกอบ:** บันทึกส่วนประกอบต่างๆ ของอาหารและเครื่องดื่มที่รับประทาน เช่น ข้าวผัดกะเพราหมูไข่ดาว ประกอบไปด้วย ข้าวสวย เนื้อหมู ใบกะเพรา พริก กระเทียม ไข่ เป็นต้น
4. **ปริมาณที่รับประทาน:** ระบุปริมาณของอาหารและเครื่องดื่มที่รับประทานโดยประมาณ เช่น ข้าวผัดกะเพราหมูไข่ดาว ประกอบด้วย ข้าวสวย 1 ถ้วยตวง เนื้อหมู 300 กรัม ไข่ไก่ 1 ฟอง โดยกำหนดปริมาณอาหารและเครื่องดื่ม เช่น

   1 ถ้วยตวง = 240 มิลลิลิตร 1 ช้อนชา = 5 มิลลิลิตร 1 ช้อนโต๊ะ = 15 มิลลิลิตร
5. **วิธีการเตรียมหรือปรุงอาหารและเครื่องดื่ม:** ระบุวิธีการประกอบอาหารและเครื่องดื่มที่รับประทาน เช่น ผัด ทอด ต้ม นึ่ง ตุ๋น ปิ้ง ย่าง แกง แช่แข็ง รับประทานสด อาหารสำเร็จรูป/อาหารกระป๋อง เป็นต้น
6. **สถานที่รับประทานอาหารและเครื่องดื่ม:** ระบุสถานที่ที่รับประทานอาหารและเครื่องดื่ม เช่น บ้าน ที่ทำงาน ร้านอาหาร เป็นต้น
7. **ผลิตภัณฑ์เสริมอาหาร:** ระบุผลิตภัณฑ์เสริมอาหารที่รับประทาน เช่น วิตามิน แร่ธาตุ พร้อมทั้งระบุขนาด จำนวน เวลาที่รับประทาน เช่น แคลเซียมคาร์บอเนต ขนาด 1 กรัม ครั้งละ 1 เม็ด หลังอาหารเช้า เป็นต้น

ภาพตัวอย่างเพื่อใช้ประมาณปริมาณอาหารที่รับประทาน

สำหรับบันทึกการบริโภคอาหาร 3 วัน

(ระบุเป็นหน่วยที่ใช้ในครัวเรือน เช่น ถ้วย ทัพพี ช้อนโต๊ะ ช้อนชา แก้ว มิลลิลิตร ชิ้น ผล ฯลฯ)

หมวดนม

นมสด

- 250 มล.

หมวดข้าว-แป้ง

หมวดเนื้อสัตว์

เนื้อไก่

- เนื้อไก่ดิบหนัก 30 กรัม
- เนื้อไก่สุกหนัก 40 กรัม

หมวดไขมัน

น้ำมัน

1 ช้อนชา หนัก 2.3 กรัม

หมวดผัก

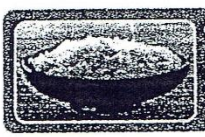
ผักกาด

- ผักกาด 2 ใบ กว้าง 10 ซม.ยาว 200 ซม. (1 ถ้วยตวง)
- ผักกาด 1 ถ้วยตวงหนัก 30 กรัม

หมวดผลไม้

กล้วยน้ำว้า

- ทั้งเปลือกหนัก 75 กรัม
- ปอกเปลือกหนัก 51 กรัม



ข้าวเจ้าสุก

- ข้าวเจ้าสุก 1 ทัพพี หนัก 80 กรัม
- ข้าวเจ้าสุก 1/3 ถ้วยตวง หนัก 55 กรัม

เลขที่โครงการวิจัย 145.1/61

วันที่รับรอง 31 ก.ค. 2561

วันหมดอายุ 30 ก.ค. 2562

แบบบันทึกการบริโภคอาหาร 3 วัน

<u>บันทึกวันที่ 1</u>

รายการอาหารที่รับประทาน วันที่.....เดือน.....ปี.....

มื้ออาหาร/เวลา	รายการอาหาร/เครื่องดื่ม	ส่วนประกอบ (คร่าวๆ)	ปริมาณที่รับประทาน	วิธีการเตรียม/ปรุงอาหาร	สถานที่รับประทานอาหาร

เลขที่โครงการวิจัย 145.1/61
วันที่รับรอง 31 ก.ค. 2561
วันหมดอายุ 30 ก.ค. 2562

แบบบันทึกการบริโภคอาหาร 3 วัน

บันทึกวันที่ 2

รายการอาหารที่รับประทาน วันที่.....เดือน.....ปี.....

มื้ออาหาร/เวลา	รายการอาหาร/เครื่องดื่ม	ส่วนประกอบ (คร่าวๆ)	ปริมาณที่รับประทาน	วิธีการเตรียม/ปรุงอาหาร	สถานที่รับประทานอาหาร

แบบบันทึกการบริโภคอาหาร 3 วัน

บันทึกวันที่ 3

รายการอาหารที่รับประทาน วันที่.....เดือน.....ปี.....

มื้ออาหาร/เวลา	รายการอาหาร/เครื่องดื่ม	ส่วนประกอบ (คร่าวๆ)	ปริมาณที่รับประทาน	วิธีการเตรียม/ปรุงอาหาร	สถานที่รับประทานอาหาร

เอกสารที่โครงการวิจัย 145.1/61
วันที่รับรอง 31 ก.ค. 2561
วันหมดอายุ 30 ก.ค. 2562

เลขที่แบบบันทึก.....

(ส่วนที่ 7)

แบบบันทึกเหตุการณ์ไม่พึงประสงค์สำหรับอาสาสมัคร (Adverse events, AE)

คำชี้แจง โปรดกรอกข้อมูลต่อไปนี้ให้ครบถ้วนตั้งแต่ครั้งแรกที่รับประทานผลิตภัณฑ์

โปรดระบุวันที่รับประทานผลิตภัณฑ์ และทำเครื่องหมาย ✓ ในช่อง "อาการผิดปกติหลังจากรับประทานผลิตภัณฑ์"

ครั้งที่รับประทาน	วัน/เดือน/ปี	อาการผิดปกติหลังจากรับประทานผลิตภัณฑ์		ลักษณะอาการผิดปกติที่เกิดขึ้นและวิธีการแก้ไขเบื้องต้น
		ไม่มี	มี	
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2				
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4				
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7				
8				
9				
10				
11				
12				
13				
14				
15				
16				

เลขที่โครงการวิจัย 145.1/61 1

วันที่รับรอง 31 ก.ค. 2561

วันหมดอายุ 30 ก.ค. 2562

ครั้งที่รับประทาน	วัน/เดือน/ปี	อาการผิดปกติหลังจากรับประทานผลิตภัณฑ์		ลักษณะอาการผิดปกติที่เกิดขึ้นและวิธีการแก้ไขเบื้องต้น
		ไม่มี	มี	
17				
18				
19				
20				
21				
22				
23				
24				
25				
26				
27				
28				
29				
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31				
32				
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36				

ครั้งที่รับประทาน	วัน/เดือน/ปี	อาการผิดปกติหลังจากรับประทานผลิตภัณฑ์		ลักษณะอาการผิดปกติที่เกิดขึ้นและวิธีการแก้ไขเบื้องต้น
		ไม่มี	มี	
37				
38				
39				
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56				

เลขที่โครงการวิจัย 145.1/61 3

วันที่รับรอง 31 ก.ค. 2561

วันหมดอายุ 30 ก.ค. 2562

ครั้งที่รับประทาน	วัน/เดือน/ปี	อาการผิดปกติหลังจากรับประทานผลิตภัณฑ์		ลักษณะอาการผิดปกติที่เกิดขึ้นและวิธีการแก้ไขเบื้องต้น
		ไม่มี	มี	
57				
58				
59				
60				
61				
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ครั้งที่รับประทาน	วัน/เดือน/ปี	อาการผิดปกติหลังจากรับประทานผลิตภัณฑ์		ลักษณะอาการผิดปกติที่เกิดขึ้นและวิธีการแก้ไขเบื้องต้น
		ไม่มี	มี	
77				
78				
79				
80				
81				
82				
83				
84				

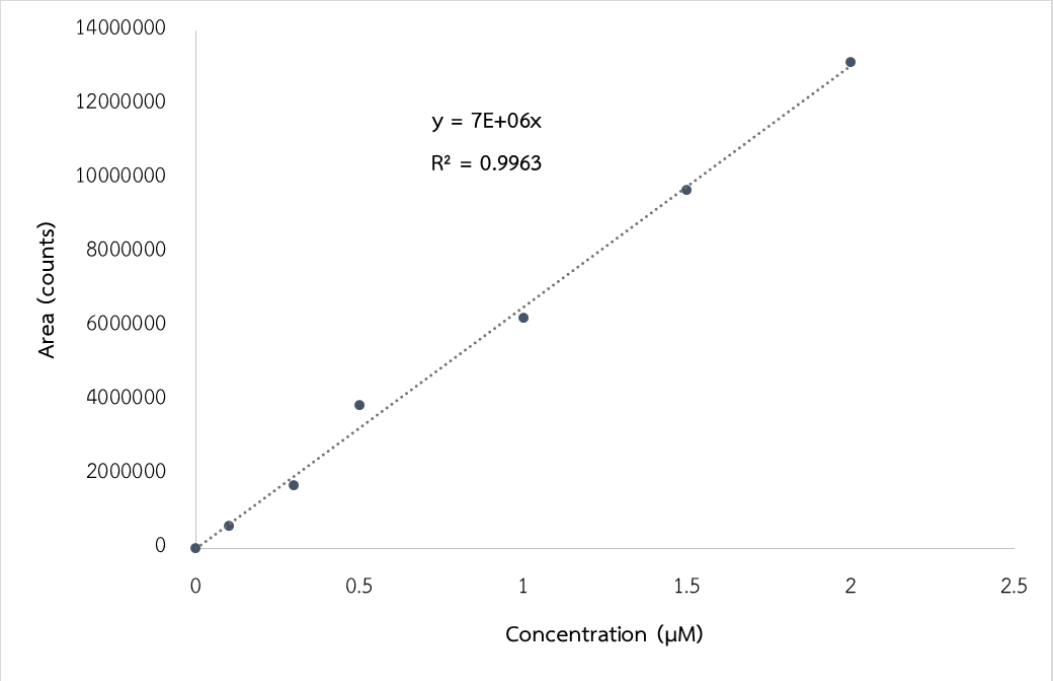
ขอบพระคุณที่ให้ความร่วมมือ

เลขที่โครงการวิจัย 145.1/61
วันที่รับรอง 31 ก.ค. 2561
วันหมดอายุ 30 ก.ค. 2562

APPENDIX D

Calibration curve to estimate serum astaxanthin concentration

Concentration of standard solution vs. AUC



VITA

NAME	Chuenjai Sratongfaeng
DATE OF BIRTH	28 August 1989
PLACE OF BIRTH	Nakhonsawan, Thailand
INSTITUTIONS ATTENDED	Faculty of Pharmacy, Silpakorn University Faculty of Pharmaceutical Sciences, Chulalongkorn University
HOME ADDRESS	Bangkok, Thailand