Effects of Mulberry (*Morus alba*) Fruits on Lipid Profiles, Antioxidant, and Inflammation status in Hypercholesterolemic Subjects

Mr. Wiroje Kaewruang

Asst. Prof. Dr. Patcharanee Pavadhgul

Asst. Prof. Dr. Akkarach Bumrungpert

Miss. Anchalee Sirikanchanarod

Presented by

Mrs. Suthira Ponjaruen

Introduction and Literature Review

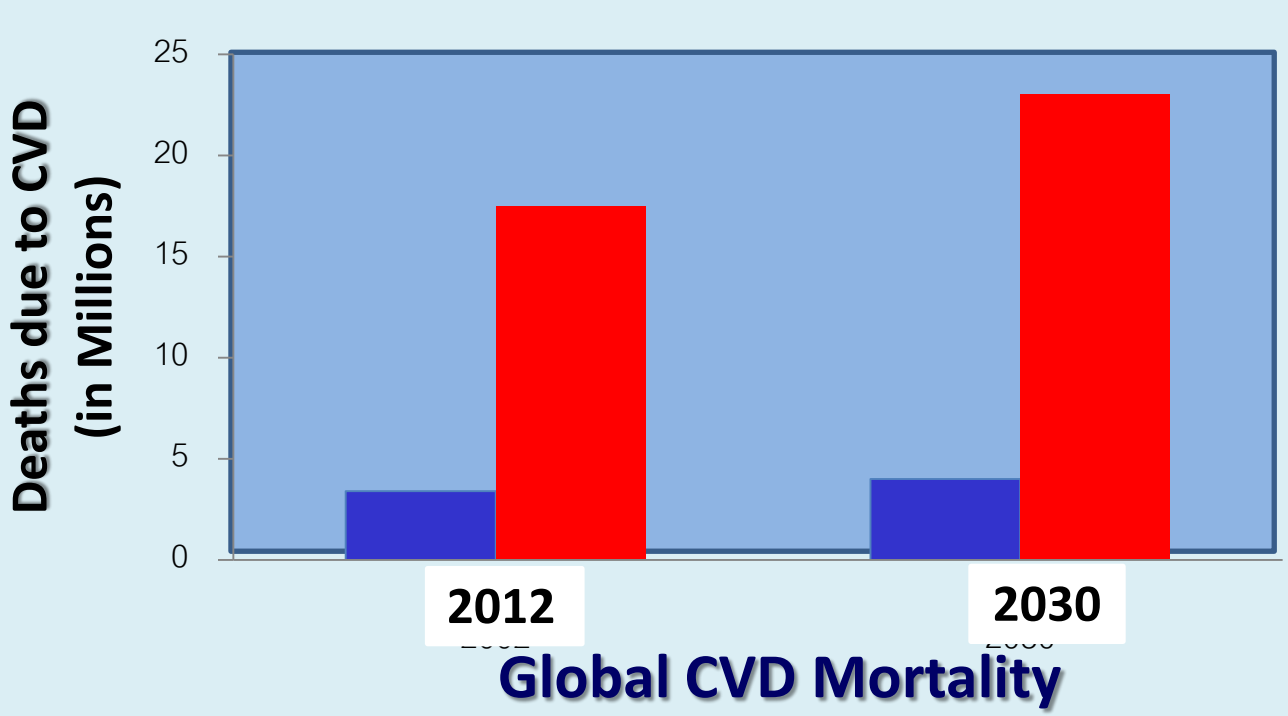
Cardiovascular disease (CVD)

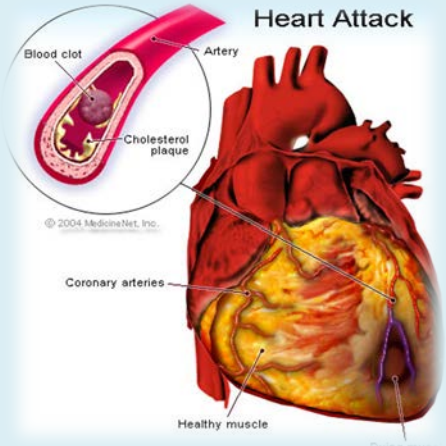
- Cardiovascular disease (CVD) is a non communicable disease, but it is an important public health problem worldwide.

- High cholesterol in blood is an important risk factor of atherosclerosis that various diseases such as coronary heart disease and stroke in the future.

Cardiovascular diseases (CVD)

In 2012, an estimated 17.5 million people died from CVD,mainly from coronary heart disease (7.4 million) and stroke(6.7 million). This number is expected to increase to 23 million people in 2030





Source: *World Health Organization. Global status report on noncommunicable diseases 2014.*

Introduction

❑ **Risk factors of cardiovascular disease**

Non-modification

- Sex
- Age
- Family history of premature CHD

Modification

- High blood cholesterol
- High blood pressure
- Diabetes
- Unhealthy diet
- Overweight/obesity
- Cigarette smoking
- Physical inactivity

Source: *World Heart Organization*

Introduction

The WHO recommends fruits and vegetables intake 400 g/day, adequate against the occurrence of chronic diseases.

Fruits and vegetables are rich in

- ✓ nutrients
- ✓ fiber
- ✓ phytochemicals
- ✓ low in calories

6

Introduction

Human intervention studies using berries fruits (fresh, or juice, or freeze-dried), or purified anthocyanin extracts

improvements in
- LDL oxidation
- lipid peroxidation
- total plasma antioxidant capacity

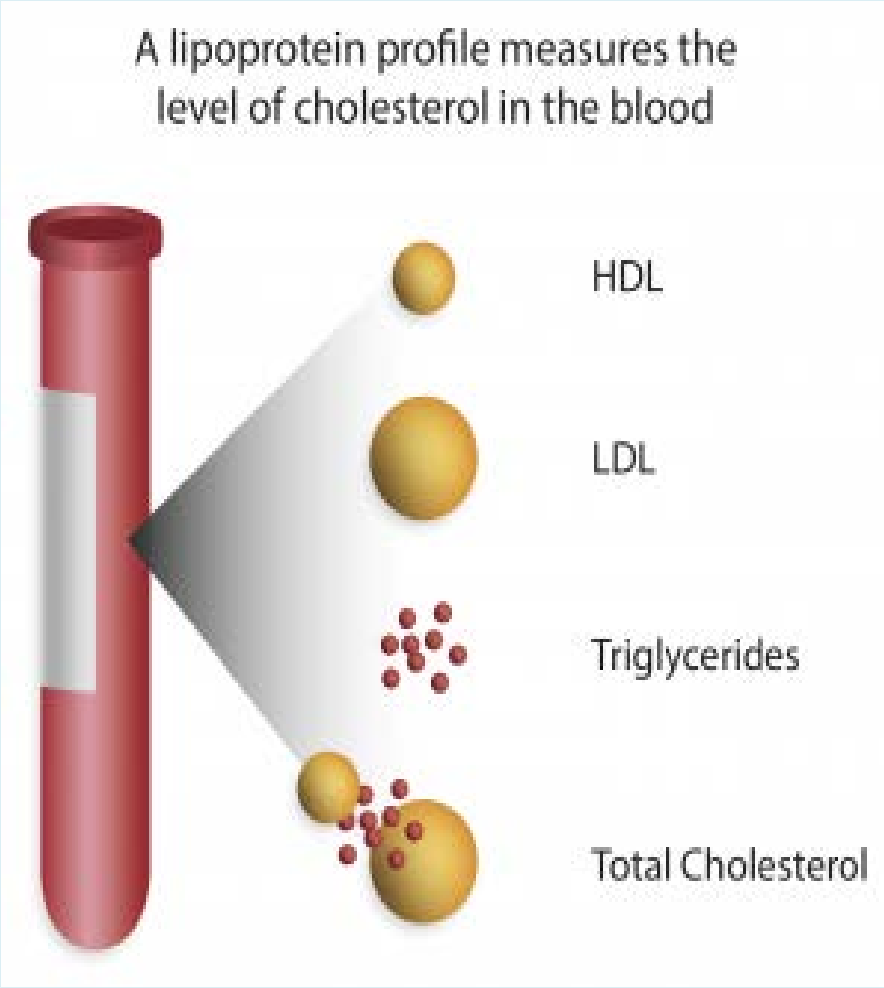
Berries are the best source
- ✓ polyphenol
- ✓ micronutrients
- ✓ fiber

Source: *Basu et al,2010*

Mulberries :

the best source of…
- ✓ polyphenol, especially anthocyanin
- ✓ micronutrients
- ✓ fiber

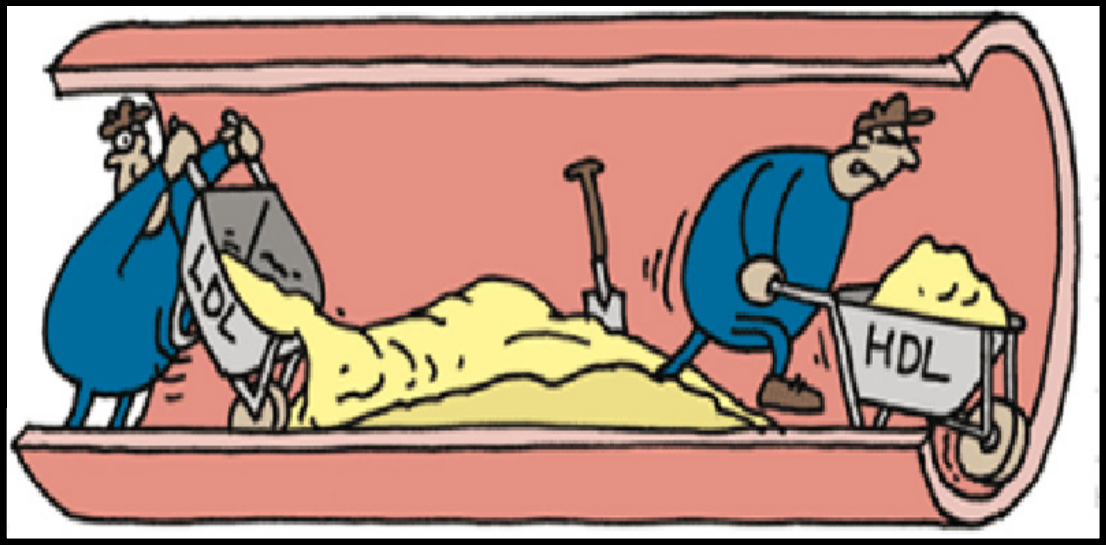
Introduction

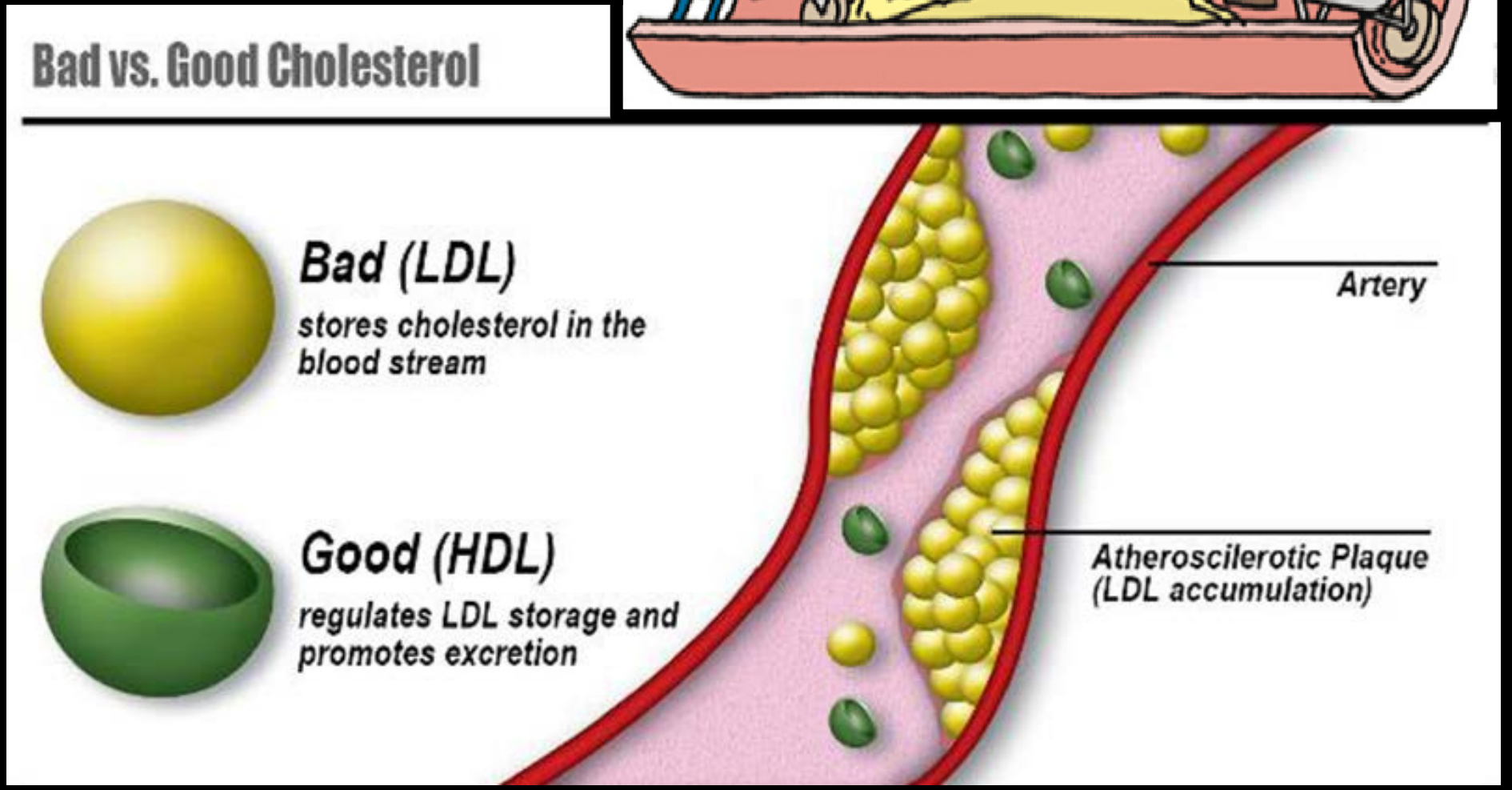


Lipid profiles:

- ✓**High- density lipoprotein cholesterol (HDL-C)**
- ✓**Low- density lipoprotein cholesterol (LDL-C)**
- ✓**Triacylglycerol (TAG)**
- ✓**Total cholesterol (TC)**

LDL-C and HDL-C ?





LDL-C and HDL-C ?

- **LDL-C : Low Density Lipoprotein (bad cholesterol) is associated with cardiovascular disease.**

- **HDL-C : High Density Lipoprotein (good cholesterol), because it prevents LDL- cholesterol and triglycerides accumulation in the arteries, cause of cardiovascular disease.**

Classification of blood lipids

Blood lipids	Concen-tration (mg/dL)	Interpretation	Blood lipids	Concen-tration (mg/dL)	Interpretation
Total cholesterol	< 200	Desirable	**HDL cholesterol**	< 40	Low
	200-239	Borderline high		≥ 60	High
	≥ 240	High			
LDL cholesterol	< 100	Optimal	**Triacylglycerol**	< 150	Normal
	100-129	Near or above optimal		150-199	Borderline high
	130-159	Borderline high		200-499	High
	160-189	High		≥ 500	Very high
	≥ 190	Very high			

Mulberry Fruit

Classification

Family: *Moraceae*

Genus: *Morus* L.

***Species*: *Morus* spp.**

There are three common mulberry species:

- *Morus* ***alba*** **(white mulberry)**
- *Morus* ***nigra*** **(black mulberry)**
- *Morus* ***rubra*** **(red mulberry)**

Mulberry Fruit Index

Red **Purple-red** **Purple-black (ripe)**

β-carotene, vitamin C

Anthocyanin

Characteristic of mulberries at degree of maturity

Sources: Aramwit *et al*. (2010), Hathaikarn *et al.* (2008)

Objectives

- To compare **lipid profiles antioxidant inflammation status** in hypercholesterolemic subjects **between** mulberry and control **group**.

- To compare **lipid profiles antioxidant inflammation status** in hypercholesterolemic subjects **before** and **after** mulberry consumption.

Materials and Methods

Study Design

- Experimental study, a randomized controlled trial

Sample size calculation

Replace a formula

$$n = \frac{(0.84 + 1.96)^2 (0.23 + 0.18)}{(2.54 - 2.19)^2}$$

$n = 26.79$

$n \sim 27$ persons

Account for drop outs 10 % = 3 persons

Total sample size 30 persons/group (Total n = 60)

The reference values for decrease LDL cholesterol, used for calculating sample size

Reference : *Alvarez-Suarez JM et al, 2014*

Recruitment

Inclusion criteria

- The subjects had hypercholesterolemia, fasting TC ≥ 200 mg/ dL, LDL-C ≥130 mg/dL.
- Age 30 to 60 years, males and females
- Willing to participate in the study.

Recruitment

Exclusion criteria

- Pre-existing disease (e.g. cardiovascular disease, diabetes mellitus, hypertension, liver, renal, thyroid disorders, cancer)
- Abnormalities in hematology (e.g. hemophilia, anemia)
- Consume antioxidant or lipid-lowering dietary supplements on a regular basis
- Use hormone replacement therapy

Recruitment

Exclusion criteria

Treatment with lipid-lowering drugs and steroidal medications

- **Pregnancy and lactation**
- **Smoking or drinking alcohol**

The freeze-dried mulberry: Chiang Mai variety

- Fresh mulberry are easily bruised.
- Antioxidant and high nutrition value that similar fresh mulberry

In the human study by Qin *et al* (2009) daily intake 320 mg anthocyanins for 12 weeks showed increased HDL-C and decreased LDL-C concentrations in dyslipidemic subjects.

$$\frac{\text{320 mg anthocyanins}}{\text{8.31 mg Freeze-dried mulberry}} \sim \text{40 g Freeze-dried mulberry}$$

Freeze-dried mulberry has anthocyanins 8.31 mg/g(Pansuwan *et al* (2010).

N=80

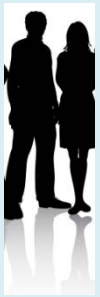
Inclusion, E xclusion criteria

N=60

Screening:

- ✓ **fast overnight** :TC, LDL-C
- ✓ **questionnaire**; general characteristics, nutrition information and lifestyle

Mulberry gr. (N = 30)



Mulberry gr. (N = 30)

Wash-out

1 week

Intervention period

6 weeks

Baseline

End of study

Control gr. (N = 30)

Control gr. (N = 28)

Experimental design (diagram)

The participants were divided into two groups which each group consisted of 30 participants.

- **Mulberry group were consumed freeze-dried mulberry which contain 45 grams per day for 6 weeks.**
- **Control group that consist of 28 participants were not consumed (2 participants were withdrawn).**

In order to follow the rules of research (compliance) samples, all participants were continuously observed by telephone and meeting every 2, 4, 6 weeks.

Methods

7 days
Prior-to experiment

Wash-out

- abstain from consuming anthocyanin rich foods
- record daily food intake
- record physical activity patterns

Baseline

Biochemical assessment

- Lipid profiles
  - TC
  - LDL-C
  - HDL-C
  - TAG

Anthropometric assessment

Within 6 weeks

Interventions

The mulberry group: 45 g freeze-dried mulberry fruit per day (320 mg anthocyanins)

The control group : no received mulberry

End of study

Biochemical assessment

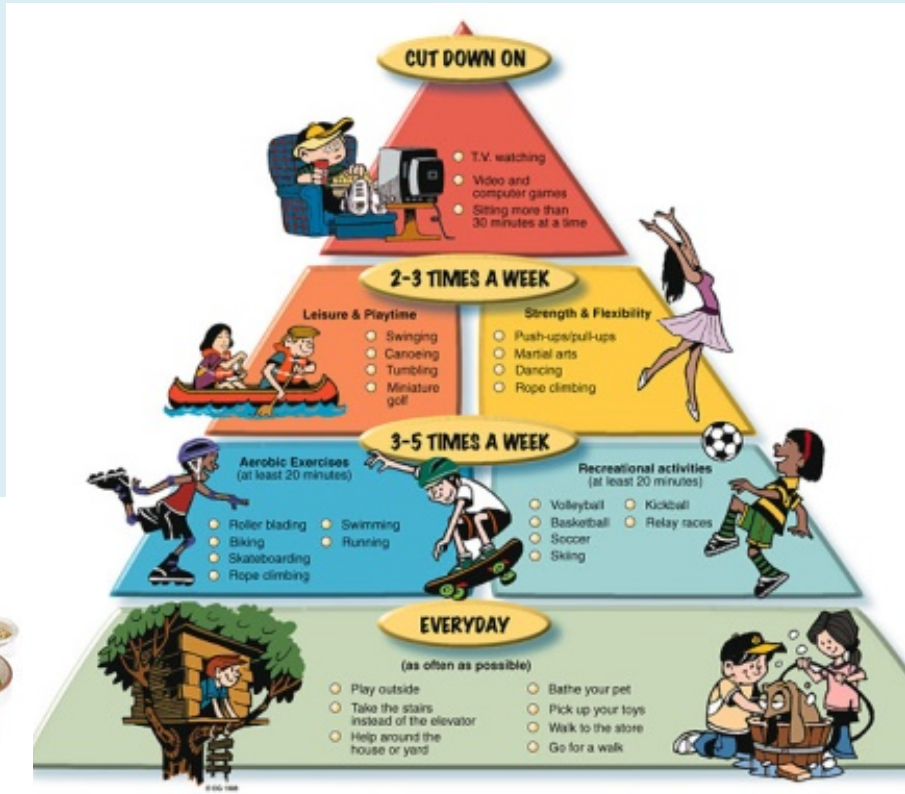
- Lipid profiles
- Antioxidants
- Inflammation

Anthropometric assessment

*during the trial, maintain their usual diet, physical activity, and lifestyle patterns

Instruments

1. General information and screening questionnaire
2. Three-day food record
3. Physical activity record



Avoid

pomegranate

Prune

Purple cabbage

eggplant

Butterfly pea

Purple sweet potato

Purple corn

Roselle juice

black sticky rice

Foods vegetables and fruits are content rich of anthocyanin.

Data collection

Biochemical assessment:

- Total cholesterol (TC)
- Triacylglycerol (TAG)
- High density lipoprotein cholesterol(HDL-C)
- Low density lipoprotein cholesterol (LDL-C)
- Antioxidant

Anthropometric assessment:

- Body weight, Height
- Body fat and visceral fat
- Body mass index (BMI)
- Waist circumference

Dietary assessment:

- Three days food record

Statistical analysis

- **Demographic data :** percentage, mean ± SD
- **Dietary nutrient intakes :**
  - Between the mulberry group and control group :
    Independent samples t - test
  - Each group at baseline, week 3, and week 6 of study:
    One-way ANOVAs
- **Lipid profiles Antioxidant and Inflammation status :**
  - Between the mulberry group and control group :
    Independent samples t - test
  - Each group (pre-posttest) :
    Paired samples t – test

Statistical significance were accepted at a probability value of < 0.05

Results

General characteristics:

General characteristics	Total (N=58)		Mulberry (N=30)		Control (N=28)		*p*-value
	N	%	N	%	N	%	
Gender							
Male	8	13.8	4	13.3	4	14.3	0.918
Female	50	86.2	26	86.7	24	85.7	
Age (years)							
30-39	11	19.0	5	16.7	6	21.4	0.268
40-49	21	36.2	9	30.0	12	42.9	
50-60	26	44.8	16	53.3	10	35.7	
Marital status							
Single	26	44.8	14	46.7	12	42.9	0.948
Married	25	43.1	12	40.0	13	46.4	
Separate	7	12.1	4	13.3	3	10.7	

General characteristics:

- gender
- age
- Marital status
- behaviors and lifestyles

no significant difference between the two groups

Anthropometry and Blood pressure

At baseline

Variables	Mulberry (N=30)	Control (N=28)	*p* - value
Anthropometry			
Body weight (kg)	58.67 ± 11.19	62.38 ± 15.67	0.301
Body mass index (kg/m^2)	23.87 ± 3.87	24.80 ± 5.41	0.454
% body fat	32.44 ± 5.28	31.34 ± 6.14	0.471
% visceral fat	7.17 ± 4.04	7.99 ± 5.93	0.621
Waist circumference (cm)	81.67 ± 9.87	82.98 ± 12.90	0.667
Blood pressure			
Systolic blood pressure (mmHg)	118.17 ± 15.18	119.00 ± 16.82	0.844
Diastolic blood pressure(mmHg)	77.37 ± 9.31	76.93 ± 10.62	0.868

At week 6

Variables	Mulberry (N=30)	Control (N=28)	*p* - value
Anthropometry			
Body weight (kg)	58.60 ± 11.05	62.72 ± 15.48	0.246
BMI (kg/m^2)	24.02 ± 3.86	24.92 ± 5.32	0.464
% body fat	32.34 ± 5.44	31.09 ± 6.81	0.563
% visceral fat	6.93 ± 3.92	8.00 ± 5.55	0.433
Waist circumference (cm)	83.22 ± 9.55	85.00 ± 12.06	0.534
Blood pressure			
Systolic blood pressure (mmHg)	115.77 ± 13.40	116.96 ± 14.55	0.745
Diastolic blood pressure (mmHg)	74.23 ± 10.44	73.79 ± 10.03	0.869

Anthropometry and blood pressure

- mulberry group
- control group

in both before and after the experiment was no difference

The effects of freeze-dried mulberry consumption on lipid profiles antioxidants and inflammation status in blood.

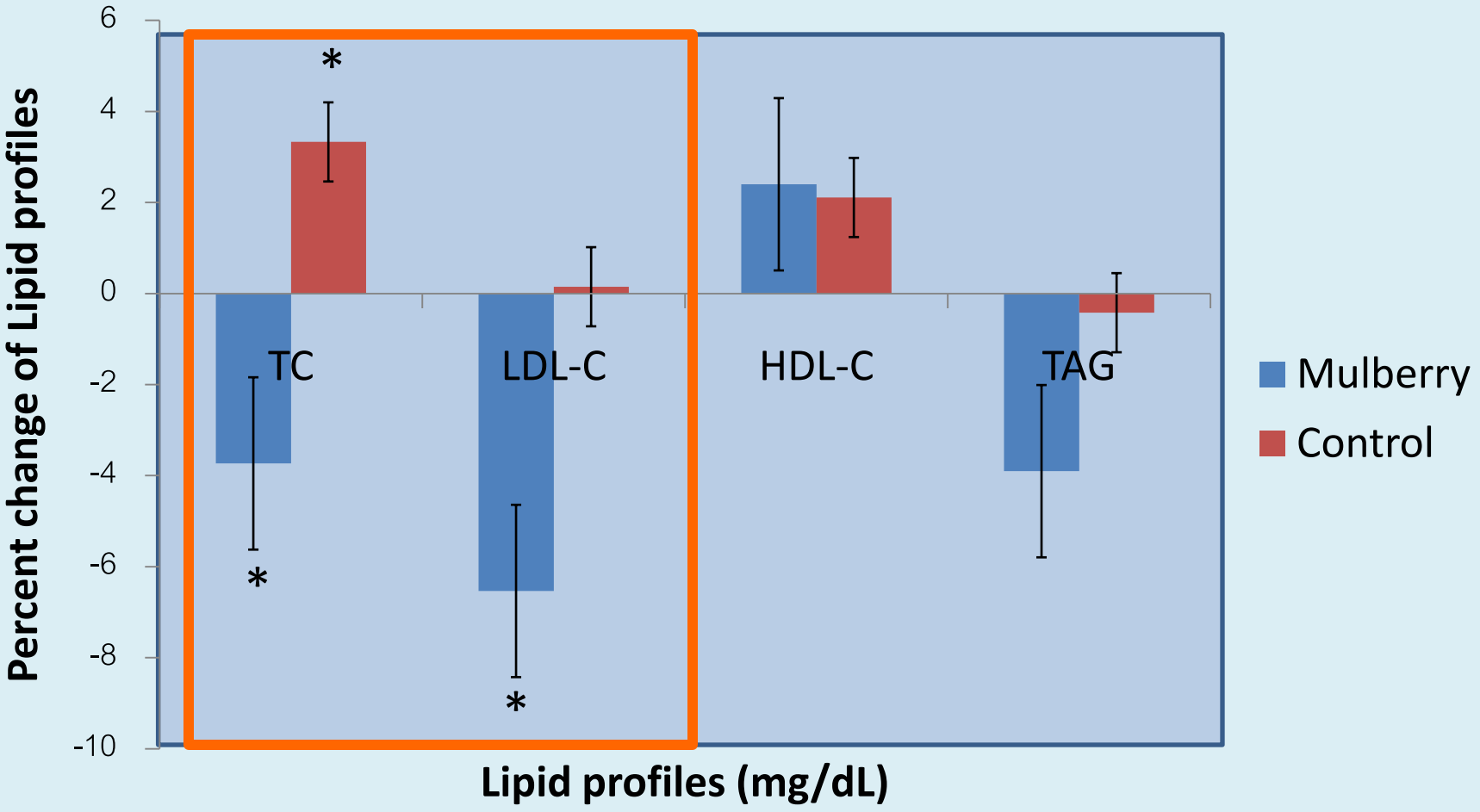
lipid profiles.

Changes of lipid profiles

Lipid profiles (mg/dL)	Group	Mulberry	Control	*p* – value
TC*	Mean change	- 9.23 ± 10.75	7.44 ± 12.13	< 0.001
	Percent change	- 3.73 ± 3.99	3.33 ± 5.07	< 0.001
LDL-C*	Mean change	- 11.40 ± 12.04	- 0.11 ± 8.77	< 0.001
	Percent change	- 6.53 ± 5.93	0.15 ± 4.90	< 0.001
HDL-C	Mean change	1.43 ± 4.96	0.75 ± 7.33	0.681
	Percent change	2.40 ± 8.60	2.11 ± 11.28	0.912
TAG	Mean change	- 7.76 ± 30.69	- 3.19 ± 25.40	0.549
	Percent change	- 3.90 ± 28.64	- 0.42 ± 25.81	0.638

*Significant differences, $p < 0.001$ assessed by independent-sample *t* test

Changes of lipid profiles



Percent change of lipid profiles between mulberry group and control group
* Significant differences, $p < 0.001$

- This study found that the consumption of freeze-dried mulberry 45 g/day for 6 weeks could significantly decrease the TC 3.73% and LDL-C 6.53%.
- no effect on TAG and HDL-C

- TC was statistically significant increased
- TAG, HDL-C , LDL-C were no statistical significant in Control group

Antioxidant and Inflammation status

Antioxidant and Inflammation status

	Group	Week0	Week 6	Mean change	Percent change
Antioxidant					
ORAC * [TE (µM) / ml (g)]	Mulberry	50.49 ± 8.89	55.16 ± 8.92	4.70 ± 4.88	10.27 ± 11.36
	Control	49.34 ± 11.91	47.25 ± 10.37	-2.09 ± 4.45	-3.16 ± 10.37
FRAP* [TE (µM) / ml (g)]	Mulberry	15.45 ± 2.33	17.06 ± 2.11	1.61± 1.58	11.39 ± 11.42
	Control	14.31 ± 2.18	14.06 ± 1.65	-0.25 ± 1.97	-0.09 ± 15.65
Inflammation hsCRP (mg/L)	Mulberry	1.75 ± 1.87	1.59 ± 1.84	-0.15± 0.77	-6.6 ± 37.49
	Control	2.30 ± 1.87	2.08 ± 1.76	-0.22 ± 0.87	-4.64 ± 34.69

Antioxidant

- In mulberry group, the antioxidant resistant value, that was analyzed by **ORAC method**, was significantly increased to 55.16 μM / ml after the 6 weeks consumption. In addition, the results from **FRAP method** was similar.

Antioxidant

- It is noticeable, in mulberry group, the antioxidant, both **ORAC** and **FRAP**, in blood was significantly increased when compared between at pre- and post-experiment.

Inflammation status

- **Inflammation status between mulberry group and control group in both before and after the experiment was no difference**

Conclusion

- Consumption 45 grams freeze-dried mulberry (325 mg anthocyanins, ~ 160 g fresh mulberry) per day for six weeks
  - Reduce **TC** (3.73%) and **LDL-C** (6.53%), respectively ($p < 0.001$)
  - No change in **HDL-C** and **TAG**
  - Increase antioxidant activity

  in hypercholesterolemic subjects

Recommendation

I. Mulberry is natural source of antioxidants and good alternative food in order to control lipid profiles to normal levels.

II. A great way to boost the fiber content in dietary intake, which benefits cardiovascular health in humans.

III. Patient's hypercholesterolemia reduced lipid lowering medications which reduce the expenses and side effects.

THANK YOU

FOR YOUR ATTENTION

Suggestion for further study

- Long term effect
- Intervention using fresh or juice mulberry in hypercholesterolemia
- In hypertension, diabetes mellitus or metabolic syndrome subjects

Author, year	Study design	Subjects	Diet	Result
Liu *et al* (2008)	experimental (in vitro) 12 weeks	Blood from healthy volunteers	MWEs (22.8 percent of anthocyanins) MACs (88.5 percent of anthocyanins)	**Inhibit** LDL oxidation **Inhibit** the death of macrophages **Decrease** foam cells formation induced by ox-LDL **Prevent** of atherosclerosis

MWEs – mulberry water extracts
MACs – mulberry anthocyaanin-rich extracts

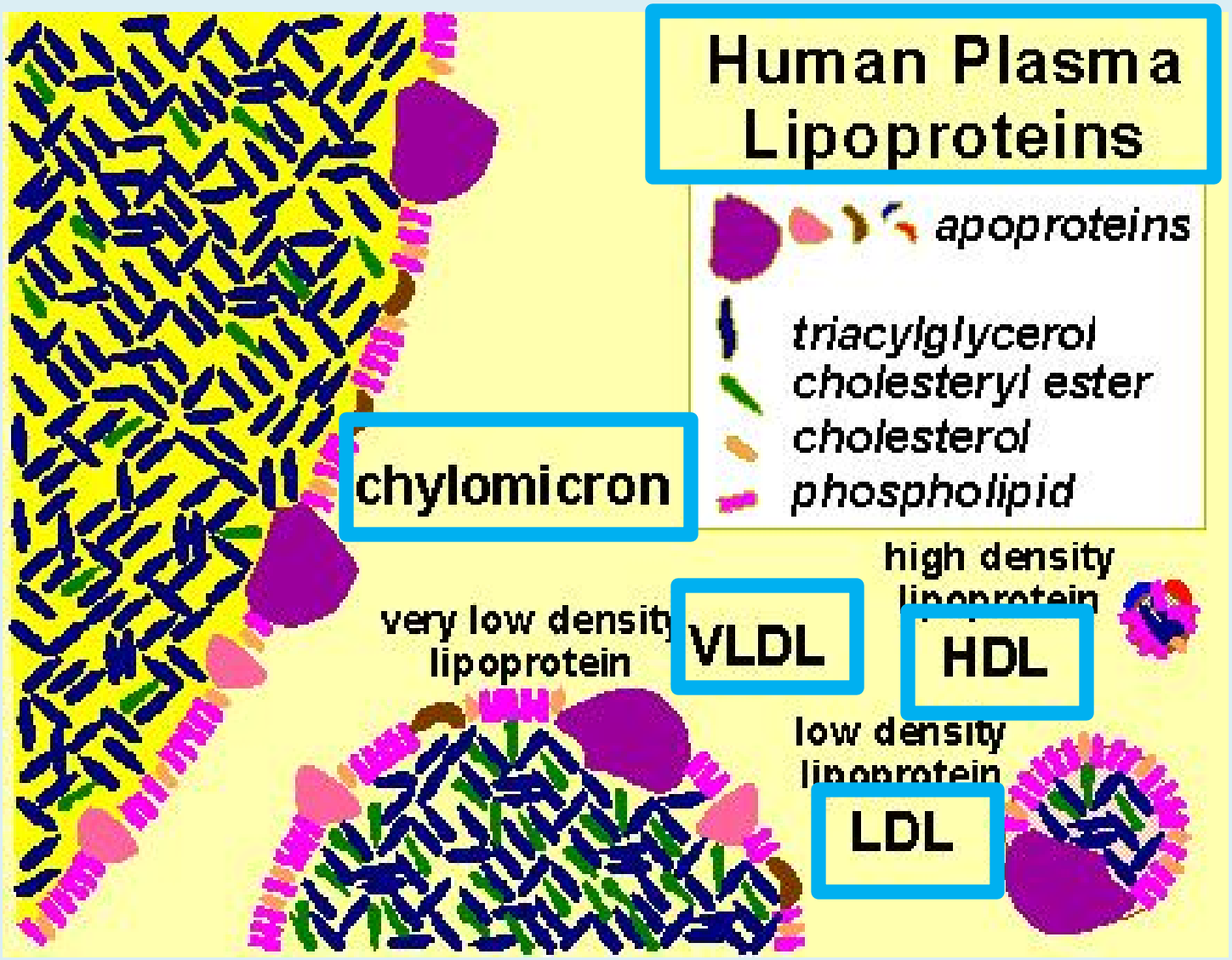
Author, year	Study design	Subjects	Diet	Result
Yang *et al*. (2010)	experimental (in vivo) 4 weeks	Hyperlipidemia rats	**(i)** normal diet **(ii)** normal diet and 5% MFP **(iii)** normal diet and 10% MFP **(iv)** high-fat diet, **(v)** high-fat diet and 5% MFP **(vi)** high-fat diet and 10% MFP	rats on normal diet and MFP **no significant** change in lipid profile in the serum and liver. rats on high-fat diet and MFP significantly ↓ **reduced** level of serum and liver TG, TC, LDL-C ↓ **reduced** TBARS ↑ **increased** HDL-C ↑ **increased** SOD, GSH-Px

MFP - mulberry fruit freeze-dried powder
TBARS - Thiobarbituric acid reactive substances
SOD – superoxide dismutase
GSH-Px – glutathione peroxidase

↑ Increase

↓ Decrease

Literature reviews



LDL-C and HDL-C ?

- **LDL (bad cholesterol) its low density Lipoprotein is associated with cardiovascular disease is the fat that can cause arteries to harden the fat LDL acts transports cholesterol come from the liver into the blood in the body if cholesterol remains in the bloodstream it accumulated on the walls of blood vessels, causing severe coronary artery stenosis solid long it will cause clogged arteries and high blood pressure as a result of ischemic heart disease kidney failure, paralysis etc.**
- **HDL stands for High Density Lipoprotein (good cholesterol), HDL high-density cholesterol is good because the arteries prevents cholesterol, triglycerides, and LDL accumulation in the arteries of HDL in the blood, it makes it more likely to be heart disease and stroke.**

Literature reviews (cont')

Harvested during: the months of December to March

Cultivated : the northern and northeast of the Thailand

Consumption: fresh mulberry, mulberry product such as mulberry juice, wine, jam, jelly, ice-cream, candy, canned food and freeze-dried


Mulberry

Table 3 Nutrient composition of fresh and freeze-dried mulberries *(Morus alba)* values in per 100 grams

Nutrient	Unit	Ripe mulberries[1]	Ripe mulberries[2]	Ripe mulberries[3]	Freeze-dried mulberries[3]
Proximate composition					
Water	g	72.95	81.72	85-88	3.5-17
Energy	kcal	96.35	67.36	43	-
Protein	g	1.68	1.55	0.50-1.40	-
Lipid	g	0.47	0.48	0.39-0.50	7.54
Carbohydrate	g	21.35	14.21	7.8-9.8	-
Fiber	g	2.03	1.47	0.9-1.7	24.3
Sugars	g	-	7.55	1.8-16.2	72.7-80.2

Sources: [1] Wasan,2545 [2]Imran, 2010 [3]Mine,2013

Table 3 Nutrient composition of fresh freeze-dried mulberries *(Morus alba)* values in per 100 grams (cont')

Nutrient	Unit	Ripe mulberries[1]	Ripe mulberries[2]	Ripe mulberries[3]	Freeze-dried mulberries[3]
Vitamins and minerals					
Calcium (Ca)	mg	0.21	576	39-443	-
Potassium (K)	mg	-	1,731	194-1,668	-
Sodium (Na)	mg	-	280	10-61	-
Magnesium (Mg)	mg	-	240	17-115	-
Iron (Fe)	mg	43.48	73	1.85-190	48.1
Phosphorus (P)	mg	0.07	-	35-247	-
Zinc (Zn)	mg	-	50.20	0.12-3.20	12.1
Vitamin A	IU	25	-	-	-
Vitamin B1	mg	50.65	-	-	-
Vitamin B2	mg	3.66	0.09	-	-
Vitamin B6	mg	930.10	-	-	-
Vitamin C	mg	4.16	15.20	11-36.40	1.20
Folic acid	mg	6.87	-	-	-
Niacin	mg	0.72	3.10	-	-
Tannin	g	1.06	-	-	-

Sources: [1] Nuipirom W,2545 [2]Imran, 2010 [3]Mine,2013

Mulberry (cont')

Table 4 Quality of fresh and freeze-dried mulberries

Quality	Fresh		Freeze-dried	
	Red	Purple-black (Mature)	Red	Purple-black (Mature)
Total polyphenol (µg/g)	1,200.49 ± 55.42	3,716.24 ± 63.83	10,074.08 ± 70.85	10,521.18 ± 96.6
Total Anthocyanin (µg/g)	150.40 ± 36.32	2,940.70 ± 60.44	3,151.51 ± 75.7	8,306.91 ± 66.8
Quercetin (µg/g)	1.03 ± 0.43	3.08 ± 0.45	5.72 ± 0.24	17.50 ± 0.50
Antioxidant activity	5.66 ± 0.82	8.45 ± 0.95	11.87 ± 1.44	13.40 ± 2.88

Source: Fresh-Nampanon H *et al*, 2008
Freeze-dried; Pansuwan S *et al* , 2007

Anthocyanin in fruits

Table 5 Anthocyanin contents in fruits origin

Fruits	Anthocyanin content (mg/ 100 grams fresh weight)
Bilberry	300-698
Blackberry	82.5-325.9
Blueberry	61.8-299.6
Cranberry	67-140
Cherry	2-450
*Mulberry	294
Raspberry	20-687
Strawberry	19-55

Sources: *Nampanon H *et al*, 2008 , Sonia de Pascual-Teresa,2010

Author, year	Study design	Subjects	Diet	Result
Basu *et al* . (2010)	Intervention study (in human) 8 weeks	Metabolic syndrome subjects (N=27, n= 15 in treatment)	four cups (50 grams freeze-dried strawberry powder) of the strawberry beverage daily (~ 500 g fresh strawberries) or placebo, equivalent amounts of fluids	↓ TC (10 %) ↓ LDL-C (11 %) ↓ small LDL particles (14 %) ↔ TG, HDL-C

N - Number
CRP - C-reactive protein
TNFα - tumour necrosis factor α

↓ **Decrease**
↔ **No significant change**

Author, year	Study design	Subjects	Diet	Result
Curtis *et al* (2009)	Double-blind,randomized,placebo-controlled trail (in human) 12 weeks	Healthy postmenopausal women (N=52)	Anthocyanins derived from elderberry 500 mg/day or placebo	↔ Biomarker of inflammatory (CRP, TNFα) ↔ Plasma lipids (TC, HDL-C, LDL-C, TG)
Zhu *et al* . (2012)	Double-blind,randomized,placebo-controlled trail (in human) 24 weeks	Hypercholesterolemic subjects (N=150)	Pure anthocyanins derived from bilberry and black currant 320 mg /day or placebo	↑ HDL-C (14 %) ↓ LDL-C (10.4 %) ↔ TC,TG, insulin

N - Number
TG – Triacylglycerol
TC – Total cholesterol

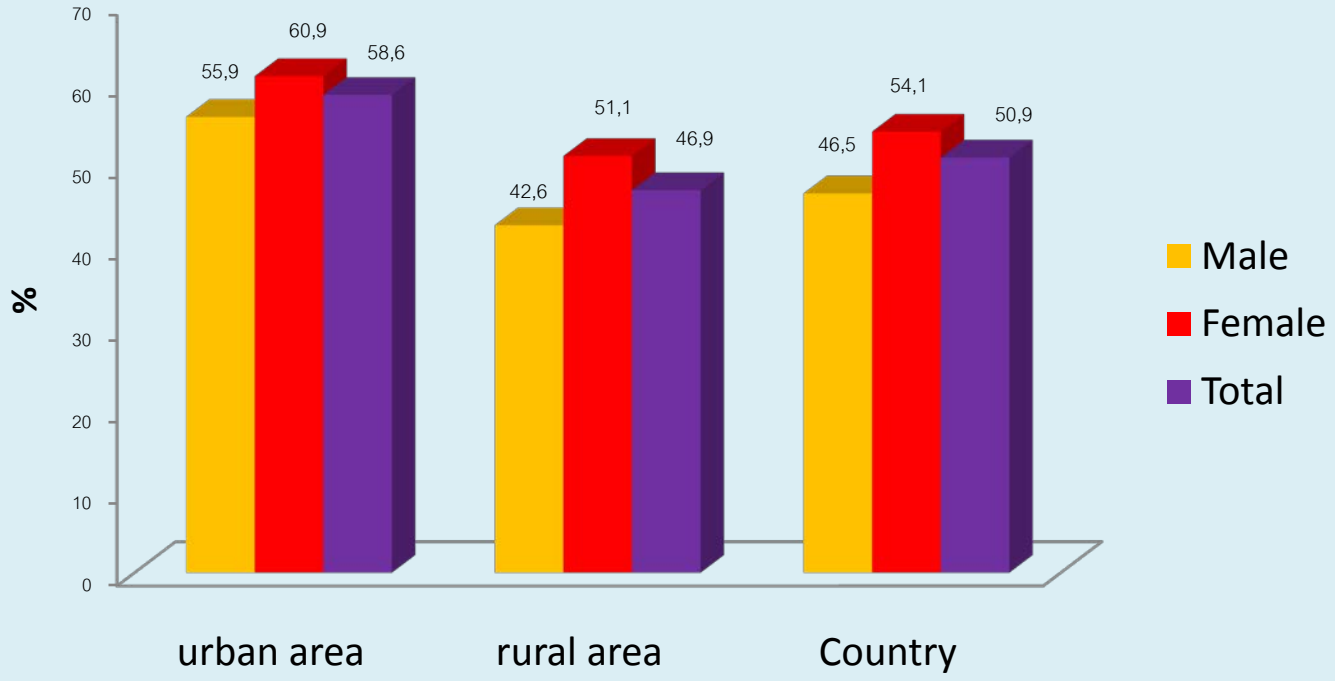
↑ **Increase**
↓ **Decrease**
↔ **No significant change**

General characteristics (cont')

General characteristics	Total (N=58)		Mulberry (N=30)		Control (N=28)		*p*-value
	N	%	N	%	N	%	
Level of education							
High school, Diploma	13	22.4	9	30.0	4	14.3	0.450
Bachelor degree	30	51.7	13	43.3	17	60.7	
Master degree or high	15	25.9	8	26.7	7	25.0	
Characteristics of work							
Sedentary	38	65.5	19	63.3	19	67.9	0.723
Moderate movement	20	34.5	11	36.7	9	32.1	
Income per month (bath)							
≤ 10,000	2	3.4	1	3.3	1	3.6	0.877
10,001 – 20,000	15	25.9	8	26.7	7	25.0	
20,001 – 30,000	21	36.2	11	36.7	10	35.7	
> 30,001	20	34.5	10	33.3	10	35.7	

Introduction (cont')

Figure 1 The prevalence of **borderline high cholesterol** (TC ≥ 200 mg/dL) in Thai people who age upper than 15 years old



Source: The forth National Health Examination Survey in 2008-2009

Study Design

- Experimental study, a randomized controlled trial

Sample size calculation

This research will calculate using the following formula:

$$n = \frac{(Z_{\beta} + Z_{\alpha/2})^2 (\sigma_1^2 + \sigma_2^2)}{(\mu_1 - \mu_2)^2}$$

$\beta = 0.20$ $Z_{\beta} = Z_{0.20} = 0.84$

$\alpha = 0.05$ $Z_{\alpha/2} = Z_{0.025} = 1.96$

Mulberries on lipid profiles, antioxidant activity, and inflammatory status

At baseline

Variables	Mulberry (N=30)	Control (N=28)	*p* - value
Lipid profiles			
TC (mg/dL)	235.07 ± 28.10	237.26 ± 32.72	0.787
LDL-C (mg/dL)	167.47 ± 29.32	166.59 ± 34.02	0.917
HDL-C (mg/dL)	61.13 ± 12.59	65.39 ± 15.86	0.261
TAG (mg/dL)	117.03 ± 41.21	100.50 ± 31.97	0.105

At week 6

Variables	Mulberry (N=30)	Control (N=28)	*p* - value
Lipid profiles			
TC (mg/dL)	225.83 ± 24.26	244.70 ± 33.12	0.019
LDL-C (mg/dL)	156.07 ± 24.70	166.48 ± 32.64	0.184
HDL-C (mg/dL)	62.57 ± 13.66	66.14 ± 15.96	0.362
TAG (mg/dL)	109.28 ± 43.65	97.31 ± 30.74	0.250

Chapter V
Discussion

Discussion

Author, year	Study design	Subjects	Diet	Result
Present study	randomized, controlled trail (in human) 6 weeks	hypercholesterolemic subjects (N=58)	45 g freeze-dried mulberry per day (325 mg anthocyanins) or control	↓ TC (3.73 %) ↓ LDL-C (6.53 %)
Qin *et al*. (2009)	Double-blind,randomized ,placebo-controlled trail (in human) 12 weeks	Dyslipidemic subjects (N=120)	Natural purified anyhocyanins derived from bilberry and black currant 320 mg / day or placebo	↑ HDL-C (13.7 %) ↓ LDL-C (13.6 %) ↓ CETP activity

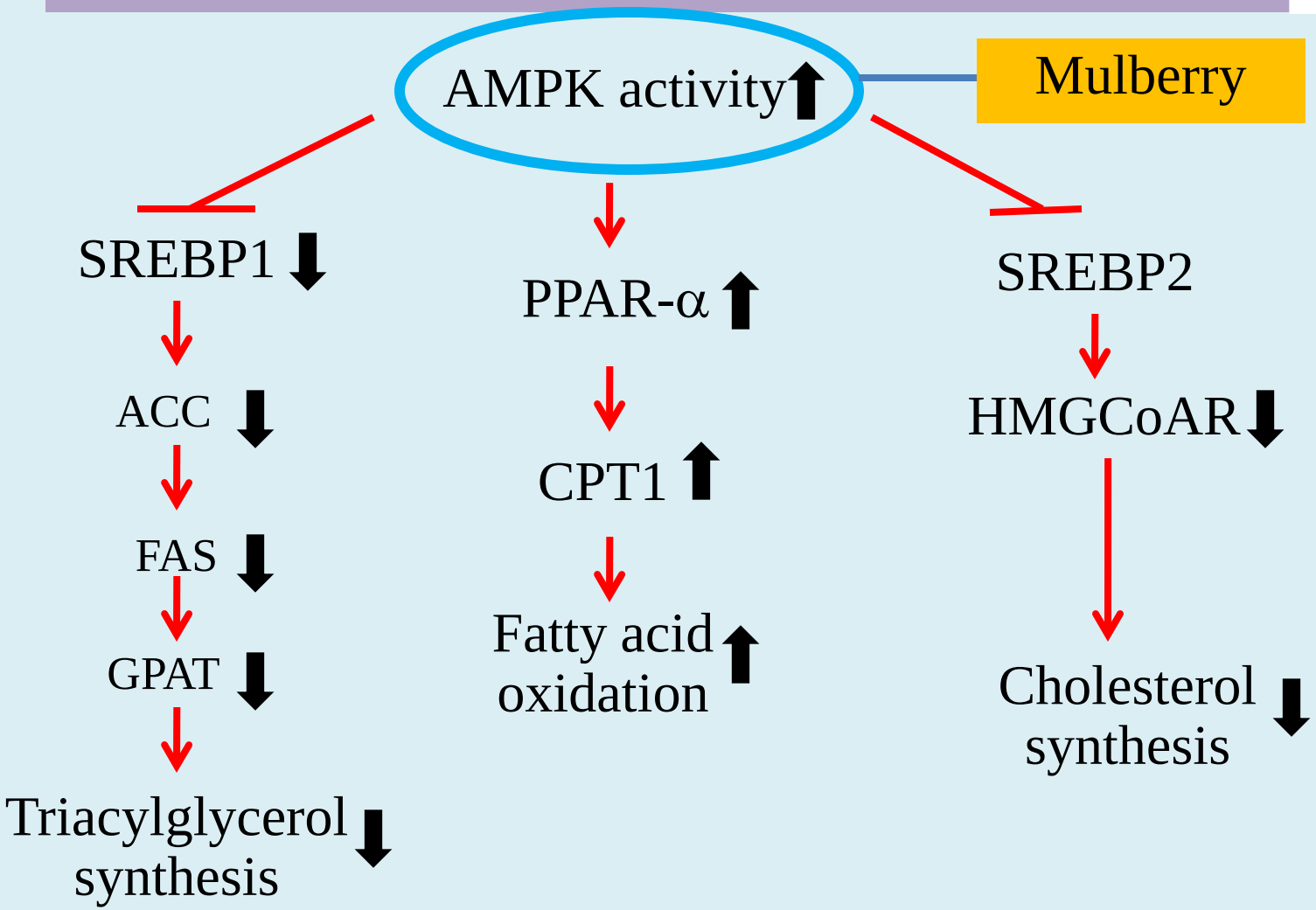
CETP – cholesteryl ester trandfer protein

Discussion

Author, year	Study design	Subjects	Diet	Result
Ou *et al* . (2011)	experimental (in vitro) 24 hour	In human hepatoma HepG2 cells	Mulberry water extracts 5.66 % ± 1.53 of anthocyanins	**Inhibition** lipogenic enzymes (FAS, ACC) **Suppressed** fatty acid synthesis. **Stimulated** fatty acid oxidation

FAS - fatty acid synthase
ACC – acetyl-CoA carboxylase

Discussion


AMPK activity ⬆
Mulberry
SREBP1 ⬇
ACC ⬇
FAS ⬇
GPAT ⬇
Triacylglycerol synthesis ⬇
PPAR-α ⬆
CPT1 ⬆
Fatty acid oxidation ⬆
SREBP2
HMGCoAR ⬇
Cholesterol synthesis ⬇

Discussion

Author, year	Study design	Subjects	Diet	Result
Duangjai *et al* . (2011)	experimental (in vitro)	Caco-2 cells	Mulberry extracts 100 µg/mL	**Blocked** the uptake of cholesterol into Caco-2 cells (about 25% inhibition)

Discussion

Mulberry Fruits

- quercetin (6.9 µg/g dry fruits)
- rutin (41.35 µg/g dry fruits)

- Wu *et al* suggest rutin cloud reduce lipid accumulation by **decreasing the activity of key enzymes** in lipid metabolism, such as ACC, FAS.

Discussion

Dietary calcium is one mineral

- **up-regulate CYP7A1** (the gene of cholesterol-7α- hydroxylase)
- **decreased** plasma **CETP** activity

Leading to reduction in the liver and plasma cholesterol level

Discussion

- The freeze-dried mulberry provide approximately 24.3 grams fiber/100 grams freeze-dried mulberry

- **In our study** may have been due to the increase in dietary fiber intake from baseline about **10 grams per day** during the **six weeks**

Discussion

- Castro *et al* following 316 of Japanese-Brazilians subjects in 7 years observed that a decrease of **12.5 mg/dL** in the serum **TC** levels for each increase of 10 grams in the consumption of dietary fiber intake.

- Streppel *et al* found that for **every 10 grams** per day increment in fiber, there was a **17%** reduction in **CHD** mortality and a **9%** reduction in **all-cause** mortality.

Discussion

The mechanisms of cholesterol-lowering effects of mulberry as follow:

- Blocked the **uptake** of cholesterol into Caco-2 cells
- Inhibited of cholesterol ester transfer protein (**CETP**) activity
- Stimulating **AMPK** pathway
- Up-regulate the gene of **CYP7A1**

Discussion

The synergistic effects of

- **Anthocyanins**
- **Polyphenols (e.g. rutin, quercetin)**
- **Fiber**

in mulberry fruits are possibly the reasons **decreasing serum TC, LDL-C** levels of hypercholesterolemic subjects.

Dietary intake (cont')

Mulberry group

	Mulberry (N = 30)			*p*-value
	Week 0	Week 3	week 6	
Energy (kcal/d)	1,265.0 ± 279.1	1,194.7 ± 302.0	1,163.7 ± 295.7	0.393
Carbohydrate				
(g/d)	188.0 ± 47.8	175.8 ± 47.0	170.1 ± 41.8	0.305
(% of energy)	59.2 ± 8.2	58.8 ± 7.3	59.1 ± 8.3	0.974
Protein				
(g/d)	43.1 ± 14.2	41.5 ± 12.7	44.1 ± 17.5	0.793
(% of energy)	13.7 ± 2.6	14.1 ± 2.3	14.7 ± 2.9	0.315
Fat				
(g/d)	38.1 ± 13.3	37.0 ± 14.1	35.4 ± 15.2	0.771
(% of energy)	26.6 ± 6.6	28.0 ± 9.0	26.2 ± 6.8	0.614
Cholesterol (mg/d)	202.4 ± 100.2	199.4 ± 118.8	195.0 ± 118.8	0.968
Fiber (g/d)	10.7 ± 6.9[a]	19.4 ± 5.9[b]	18.4 ± 4.3[b]	< 0.001*

Dietary intake (cont')

Control group

	Control (N = 28)			*p*-value
	Week 0	**Week 3**	**week 6**	
Energy (kcal/d)	1,243.9 ± 288.6	1,209.7 ± 256.4	1,183.3 ± 230.9	0.702
Carbohydrate				
(g/d)	189.0 ± 49.1	181.9 ± 53.0	171.2 ± 44.2	0.422
(% of energy)	58.9 ± 7.4	59.0 ± 8.2	57.7 ± 8.2	0.796
Protein				
(g/d)	43.1 ± 12.2	41.9 ± 10.5	41.7 ± 11.8	0.890
(% of energy)	14.0 ± 3.1	14.0 ± 2.9	14.8 ± 4.1	0.650
Fat				
(g/d)	36.5 ± 13.0	37.8 ± 12.3	37.2 ± 10.8	0.966
(% of energy)	25.5 ± 6.0	27.2 ± 6.3	28.1 ± 6.1	0.317
Cholesterol (mg/d)	203.1 ± 94.3	201.6 ± 87.7	195.9 ± 92.8	0.957
Fiber (g/d)	8.0 ± 4.9	8.3 ± 3.7	7.7 ± 3.8	0.865

Dietary intake

		Mulberry	Control	*p* - value
Energy (kcal/d)	Week 0	1,265.0 ± 279.1	1,243.9 ± 288.6	0.783
	Week 6	1,163.7 ± 295.7	1,183.3 ± 230.9	0.785
Carbohydrate				
(g/d)	Week 0	188.0 ± 47.8	189.0 ± 49.1	0.939
	Week 6	170.1 ± 41.8	171.2 ± 44.2	0.925
(% of energy)	Week 0	59.2 ± 8.2	58.9 ± 7.4	0.872
	Week 6	59.1 ± 8.3	57.7 ± 8.2	0.542
Protein				
(g/d)	Week 0	43.1 ± 14.2	43.1 ± 12.2	0.984
	Week 6	44.1 ± 17.5	41.7 ± 11.8	0.560
(% of energy)	Week 0	13.7 ± 2.6	14.0 ± 3.1	0.725
	Week 6	14.7 ± 2.9	14.8 ± 4.1	0.977

Dietary intake (cont')

		Mulberry	Control	*p* - value
Fat				
(g/d)	Week 0	38.1 ± 13.3	36.5 ± 13.0	0.659
	Week 6	35.4 ± 15.2	37.2 ± 10.8	0.636
(% of energy)	Week 0	26.6 ± 6.6	25.5 ± 6.0	0.543
	Week 6	26.2 ± 6.8	28.1 ± 6.1	0.286
Cholesterol (mg/d)	Week 0	202.4 ± 100.2	203.1 ± 94.3	0.979
	Week 6	195.0 ± 118.8	195.9 ± 92.8	0.973
Fiber (g/d)	Week 0	10.7 ± 6.9	8.0 ± 4.9	0.096
	Week 6	18.4 ± 4.3	7.7 ± 3.8	< 0.001*

Behaviors and lifestyles

Behaviors and lifestyles	Total (N=58)		Mulberry (N=30)		Control (N=28)		*p*-value
	N	%	N	%	N	%	
Meals per day							
2	7	12.1	6	20.0	1	3.6	0.089
3	46	79.3	22	73.3	24	85.7	
4-5	5	8.6	2	6.7	3	10.7	
Source of meals							
Cooking at home	17	29.3	8	26.7	9	32.1	0.614
Eating outside	11	19.0	6	20.0	5	17.9	
Ready to eat foods	29	50.0	15	50.0	14	50.0	
Frozen food products	1	1.7	1	3.3	-	-	
Exercise							
Never	34	58.6	15	50.0	19	67.9	0.177
Yes							
1-2 time/week	17	29.3	9	30.0	8	28.6	
3-4 time/week	6	10.3	5	16.7	1	3.6	
≥ 4 time/week	1	1.7	1	3.3	-	-	

1. General information and screening questionnaire

แบบสอบถามงานวิจัย

เรื่อง “ผลของการบริโภคผลหม่อนต่อระดับไขมันในเลือด ในผู้ที่มีระดับไขมันในเลือดสูง”

คำชี้แจงในการตอบแบบสอบถาม

แบบสอบถามชุดนี้มีทั้งหมด 3 ส่วน คือ

ส่วนที่ 1 ข้อมูลส่วนบุคคลทั่วไป

ส่วนที่ 2 ข้อมูลพฤติกรรมสุขภาพ

ส่วนที่ 3 ข้อมูลการตรวจร่างกายและผลการตรวจทางห้องปฏิบัติการ

2. Three-day food record

Code.....

แบบบันทึกอาหารบริโภค

บันทึกวัน [ ] อาทิตย์ [ ] จันทร์ [ ] อังคาร [ ] พุธ [ ] พฤหัสบดี [ ] ศุกร์ [ ] เสาร์

วันที่.....

มื้อ/รายการอาหาร	ชนิดของอาหารที่เป็นส่วนประกอบ	ปริมาณที่บริโภค								หมายเหตุ	Food Code
		ข้าว (ทัพพี)	เนื้อสัตว์ (ช้อนกินข้าว)	ผัก (ทัพพี)	ผลไม้ (ส่วน)	ไขมัน (ช้อนชา)	น้ำตาล (ช้อนชา)	นม (กล่อง/รสชาติ/ยี่ห้อ)	อื่น ๆ (ขนาด/จำนวน/ส่วน*)		

หมายเหตุ *ขนาด จำนวน และส่วน เช่น สังขยา 1 ชิ้น ขนาด 2x2 นิ้ว

คู่มือการบันทึกอาหารบริโภค

การบันทึกรายละเอียดของอาหารที่บริโภคในแต่ละวัน จะช่วยให้ท่านทราบเกี่ยวกับปริมาณและพลังงานที่ท่านบริโภคว่ามากน้อยเพียงใด ซึ่งจะเป็นเครื่องมือที่ช่วยในการควบคุมการบริโภคอาหารได้ ดังนั้นจึงขอให้ท่านบันทึกรายละเอียดของอาหารที่บริโภค ทั้งชนิด และปริมาณของอาหารที่บริโภคตามจริง โดยมีคำแนะนำในการบันทึกอาหารบริโภค ดังนี้

1. การบันทึกอาหารของท่านในครั้งนี้ ขอให้ท่านทำการบันทึกทั้งหมด 3 วัน/1 สัปดาห์ (วันธรรมดา 2 วัน และวันหยุด 1 วัน)
2. การบันทึกควรจะทำทันทีหลังรับประทานอาหารเสร็จในแต่ละมื้อ หรือแต่ละครั้งของการรับประทานอาหาร (ถ้าเป็นไปได้) เพื่อป้องกันการลืม
3. การบันทึกอาหารบริโภคให้ทำเฉพาะในวันที่ท่านมีการรับประทานอาหารตามปกติ ถ้าท่านป่วยหรือมีงานเลี้ยง ไม่ต้องทำการบันทึกในวันนั้น
4. การบันทึกอาหารที่ท่านรับประทาน ขอให้บันทึกอย่างละเอียด โดยบอกชนิดและปริมาณของอาหารนั้นให้ชัดเจน

3. Physical activity record

Code.....

แบบบันทึกกิจวัตรประจำวัน

วันที่.....

เวลา	กิจกรรมที่ทำ	ระเวลาที่ใช้ (นาที)