THE COMPARISON BETWEEN SERUM VITAMIN B12 CONCENTRATIONS IN ADULTS WITH AND WITHOUT METABOLIC SYNDROME

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MASTER OF SCIENCE
IN
ANTI-AGING AND REGENERATIVE MEDICINE

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Ajirapa Bussaracom

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ABSTRACT

The metabolic syndrome is defined as several developed cardiovascular risk factors, such as insulin resistance, obesity, dyslipidemia, and hypertension. Epidemiological data from countries around the world show that people in almost every age group can have vitamin B12 deficient, especially patients with type 2 diabetes. Several studies have found that decreased serum vitamin B12 concentration are more common in metabolic syndrome individuals. There is evidence suggests that low status of vitamin B12 may lead to adiposity, dyslipidemia, vascular endothelial dysfunction, glucose intolerance, and insulin resistance, which have been involved in the pathogenesis of metabolic syndrome.

Objectives: This study was aimed to compare the serum vitamin B12 concentrations between in adults with and without metabolic syndrome and compare the serum vitamin B12 concentrations between metabolic syndrome parameters including fasting blood sugar and lipid profiles.

Material and Methods: The method was conducted in 52 participants separated into 2 groups as metabolic syndrome and non-metabolic syndrome with a cross-sectional study design. Subjects were measured the body composition (anthropometric

measurements) and collected intravenous blood sample (20 mL) by trained medical staffs after a 10- to 12-hour fast.

Results: The result of this study is that adults with metabolic syndrome and non-metabolic syndrome have no difference in serum vitamin B12 concentrations, according to NCEP ATPIII criteria, using Mann–Whitney U test ($p<0.05$). However, there was a significant difference in serum vitamin B12 concentrations and total cholesterol levels. Serum vitamin B12 in subjects with high cholesterol level (≥200 mg/dL), which mainly comes from patients with dyslipidemia, was lower than subjects with normal cholesterol level (<200 mg/dL).

Conclusion: In conclusion, adults with metabolic syndrome and non-metabolic syndrome have no difference in serum vitamin B12 concentrations. Though, serum vitamin B12 concentrations are significantly different between subjects with high cholesterol and normal cholesterol levels.

Keywords: Metabolic Syndrome, Fasting Blood Sugar, Lipid Profiles, Vitamin B12

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CHAPTER 1

INTRODUCTION

1.1 Background

Nowadays, the environment, society, and way of life are all changing significantly. Regular behaviors, such as low physical activity, bad eating habits, smoking, and alcohol drinking, as well as non-modifiable characteristics, such as age, sex, hormonal change, and socioeconomic level; work, employment, money, or education, are all enhance metabolic syndrome (MS) (Galassi et al., 2006).

The association of many recognised cardiovascular risk factors, including insulin resistance, obesity, dyslipidemia, and hypertension, is referred to as the metabolic syndrome. (Huang, 2009). In Thailand, according to the modified NCEP-ATP III criteria, metabolic syndrome was shown to be prevalent in 15.2% of the population, with 25.8% of men and 8.2% of women in a study of Thai professionals and office workers (Lohsoonthorn et al., 2007).

Cobalamin, known as vitamin B12, is a necessary water-soluble mineral that is produced in the stomach of animals. Cobalamin absorption consists of several steps from oral mucosa, gastric intrinsic factor trough terminal ileal. In humans, cobalamin is a cofactor for two enzymes: methyl malonyl-CoA mutase, which synthesises the citric acid cycle, and methionine synthase, which converts homocysteine to methyl tetrahydrofolate. These pathways are crucial for immunological response, mitochondrial metabolism, maintaining the integrity of DNA and the myelin sheath surrounding neurons, and synthesising neurotransmitters, involved in both proper blood cell production and neurological activities (Berger et al., 2022).

Vitamin B12 levels can be reduced in elderly, patients with chronic renal or liver disease, gastric surgery, small intestinal disorders, and with the use of drugs such as proton pump inhibitors, metformin. Decreased in concentration of vitamin B12 are

associated with hematologic and neurological symptoms and may be a risk factor in cardiovascular disease (Romain et al., 2016).

Epidemiological data from several countries show that patients with diabetes mellitus (DMT2) can have vitamin B12 deficiency (Guney et al., 2016). Several studies have found that decreased serum vitamin B12 concentration are frequent in metabolic syndrome patients. There is evidence suggests that low status of vitamin B12 may lead to adiposity, dyslipidemia, vascular endothelial dysfunction, glucose intolerance, and insulin resistance, which have been involved in the pathogenesis of metabolic syndrome (Zhu et al., 2023). Besides, decreased in vitamin B12 concentrations in the body also effects on cellular metabolism in cytosols, as well as accumulation of fatty acids and cholesterol during metabolism in mitochondria (Lyon et al., 2020).

However, the vitamin B12 concentrations database in Thailand is quite insufficient, further research is suggested.

1.2 Objectives

1.2.1 To compare the serum vitamin B12 concentrations between in adults with and without metabolic syndrome.

1.2.2 To compare the serum vitamin B12 concentrations between metabolic syndrome parameters including fasting blood sugar and lipid profiles.

1.3 Research Hypothesis

1.3.1 Adults with metabolic syndrome have lower concentrations of serum vitamin B12 than non-metabolic syndrome.

1.3.2 Serum vitamin B12 concentrations are lower in adults with high fasting blood sugar (type 2 diabetes mellitus) and abnormal lipid profiles (dyslipidemia).

1.4 Conceptual Framework

Decreased levels of serum vitamin B12 effected cellular metabolisms that may be led to obesity, dyslipidemia, vascular endothelial dysfunction, hypertension, and insulin resistance which are essential risk factors for metabolic syndrome.

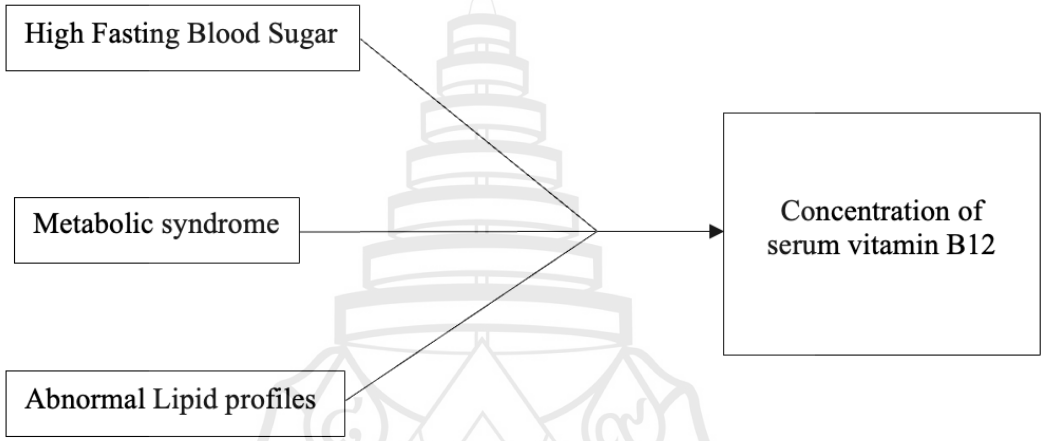


Figure 1.1 Conceptual framework

1.5 The Scope of the Study

This study included participants aged more than 45 years old who were willing to participate in February 2024, most participants were nursing college staffs from Boromarajonani College of Nursing, Nonthaburi, Thailand. All subjects were divided as a group of metabolic syndrome and non-metabolic syndrome.

The anthropometric measurements (body composition) data were assessed including weight (kg), height (cm), BMI (kg/m^2), waist circumference (cm), hip circumference (cm), waist hip ratio and blood pressure (mmHg).

The biochemical measurements (blood analyses) were performed at Bangkok Medical Laboratory (BML).

1.6 Definition

1.6.1 Obesity: According to World Health Organization (WHO) (2000) obesity can be classified by BMI as

obese I (25-29.9 kg/m^2)

obese II (≥30 kg/m^2)

overweight (23–24.9 kg/m^2).

1.6.2 BMI: The body mass index (BMI) is calculated by the ratio of body weight (kg) and the square of the height (m2).

1.6.3 Metabolic syndrome (MS): According to the Grundy et al. (2005), the parameters of MS include more than three of five following criteria:

1. Fasting blood glucose ≥ 100 mg/dL
2. Triglyceride level ≥ 150 mg/dL
3. HDL-Cholesterol level < 40 mg/dL in men and < 50 mg/dL in women
4. Blood pressure ≥ 135/85 mmHg
5. Waist circumference ≥ 90 cm for men and ≥ 80 cm for women, according to WHO (2000) are considered obesity.

1.6.4 Vitamin B12: An essential nutrient for cellular metabolism, involved energy production and epigenetic modulation processes including DNA methylation, synthesis, and repair (Sun et al., 2019).

1.6.5 Serum vitamin B12 concentrations: Using the electro-chemiluminescence immunoassay method (ECLIA) conducted by Bangkok Medical Laboratory (BML). Accepted normal range of vitamin B12 is 197-771 (pg/ml) (Saraswathy et al., 2018).

1.6.6 Fasting blood sugar: collected by trained medical staffs, stored at room temperature, and were obtained after a 10- to 12-hour fast. Cut off at ≥100 mg/dL, according to the NCEP ATP III criteria.

1.6.7 Lipid profiles: collected by trained medical staffs, stored at room temperature, and were obtained after a 10- to 12-hour fast. Cut off at

1. Total cholesterol ≥ 200 mg/dL
2. Triglyceride ≥ 150 mg/dL
3. HDL-cholesterol < 40 mg/dL in men and < 50 mg/dL in women,

4. LDL-cholesterol ≥ 130 mg/dL,

according to the NCEP ATP III criteria.

1.6.8 Adults: Participants aged 45 years old or more, according to study in Thailand by Konoknan Somnuk, which discovered that adults aged 45 and beyond are 2.35 times more likely to acquire metabolic syndrome than younger individuals (95% CI: 1.27–4.33) (Somnuk, 2020).

CHAPTER 2

LITERATURE REVIEW

In this research, the studies and documents were analyzed and separated into three parts.

1. Metabolic syndrome (MS)
2. Vitamin B12 (Cobalamin)
3. Relationships between metabolic syndrome and vitamin B12

2.1 Metabolic Syndrome

2.1.1 Definitions

Metabolic syndrome (MS) is the combination of multiple established cardiovascular risk factors, these medical conditions are associated and have common underlying mediators, processes, and pathways (Huang, 2009). MS is consisted of pathological metabolic disorders which include insulin resistance, central or abdominal obesity, dyslipidemia, and hypertension. (Grundy et al., 2005). According to WHO (2000), obesity is defined as waist circumference of ≥90 cm in men and ≥80 cm in women. According to the National Cholesterol Education Program Adult Treatment Panel III (NCEP ATPIII) criteria, the parameters of MS include in fasting blood glucose ≥100 mg/dl, triglyceride level ≥150 mg/dl, and high-density lipoprotein-cholesterol (HDL-C) <40 mg/dl in men and <50 mg/dl in women.

Metabolic syndrome has been associated with type 2 diabetes mellitus and cardiovascular disease, with an estimated 2 and 5 times greater incidence risk, respectively (Cornier et al., 2008). Statins, antihypertensives, and antidiabetic medications are among the many pharmaceutical medicines currently on the market to treat the many components of MS. Meanwhile, prescribed nutritional supplements such as folic acid and vitamin B12 are available and may help with the treatment.

Table 2.1 Definitions of metabolic syndrome

	NCEP ATP III (Grundy et al., 2005)	**WHO (2000)**	**EGIR 1999 (Grundy et al., 2005)**	**IDF 2005 (Grundy et al., 2005)**
Absolutely required	None	Insulin resistance* (IGT, IFG, T2D or other evidence of IR)	Hyperinsulinemia‡ (plasma insulin >75th percentile)	Central obesity (waist circumference§): ≥94 cm (M), ≥80 cm (F)
Criteria	Any three of the five criteria below	Insulin resistance or diabetes, plus two of the five criteria below	Hyperinsulinemia, plus two of the four criteria below	Obesity, plus two of the four criteria below
Obesity	Waist circumference: >40 inches (M), >35 inches (F)	Waist/hip ratio: >0.90 (M), >0.85 (F); or BMI >30 kg/m^2	Waist circumference: ≥94 cm (M), ≥80cm (F)	Central obesity already required
Hyperglycemia	Fasting glucose ≥100 mg/dl or Rx	Insulin resistance already required	Insulin resistance already required	Fasting glucose ≥100 mg/dl
Dyslipidemia	TG ≥150 mg/dl or Rx	TG 150 mg/dl or HDL-C: <35 mg/dl (M), <39 mg/dl (F)	TG ≥177 mg/dl or HDL-C <39 mg/dl	TG ≥150 mg/dl or Rx
Dyslipidemia (second, separate criteria)	HDL cholesterol: <40 mg/dl (M), <50 mg/dl (F); or Rx			HDL cholesterol: <40 mg/dl (M), <50 mg/dl (F); or Rx
Hypertension	>130 mmHg systolic or >85 mmHg diastolic or Rx	≥140/90 mmHg	≥140/90 mmHg or Rx	>130 mmHg systolic or >85 mmHg diastolic or Rx
Other criteria		Microalbuminuria†		

Note −*IGT, impaired glucose tolerance; IFG, impaired fasting glucose; T2D, type 2 diabetes; IR, insulin resistance; other evidence includes euglycemic clamp studies.

−†Urinary albumin excretion of 20 μg/min or albumin-to-creatinine ratio of 30 mg/g.

−‡Reliable only in patients without T2D.

−§Criteria for central obesity (waist circumference) are specific for each population; values given are for European men and women.

−Rx, pharmacologic treatment.

Source Huang (2009)

2.1.1.1 National Cholesterol Education Program Adult Treatment Panel III (Grundy et al., 2005)

Metabolic syndrome (MS) is defined as if patient has more than three of five criteria:

1. High fasting blood sugar: ≥100 mg/dL
2. Hypertriglyceridemia: ≥150 mg/dL
3. Low High-Density

Lipoprotein Cholesterol (HDL-C): <40 mg/dL in men and <50 mg/dL in women

4. High blood pressure: >130/85 mmHg
5. Abdominal obesity: WC >102 cm in men and >88 cm in women

2.1.1.2 World Health Organization: WHO (2000)

Metabolic syndrome (MS) is defined as insulin resistance (IR), diabetic mellitus (DM), impaired glucose tolerance (IGT), and two or more of the following factors:

1. Raised arterial pressure: ≥140/90 mmHg
2. Raised plasma triglyceride: ≥ 150 mg/dl or low HDL-C: <35 mg/dl in men and <39 mg/dl in women
3. Central obesity: waist hip ratio (WHR) >0.9 in men and >0.85 in women and/or body mass index (BMI) >25 kg/m^2
4. Microalbuminuria: urinary albumin excretion rate ≥ 20 μgm/minute or albumin/creatine ratio ≥ 30 μgm/mg

2.1.1.3 EIGR definition the diagnostic criteria (Grundy et al., 2005)

Including two additional criteria from the following list in addition to increased plasma insulin (>75th percentile):

1. Abdominal obesity: waist circumference (WC) ≥94 cm in men and ≥80 cm in women
2. Hypertension: ≥140/90 mm of Hg or on antihypertensive treatment
3. Elevated triglycerides: ≥177 mg/dl or reduced HDL-C: <39 mg/dl
4. Elevated plasma glucose: impaired fasting glucose (IFG) or impaired glucose tolerance (IGT)

2.1.1.4 International Diabetes Federation (IDF) (Grundy et al., 2005)

This definition of MS includes central obesity (based on race and gender-specific cutoffs) and any two of the following four parameters:

1. Raised triglycerides: ≥150 mg/dl or history of specific treatment for this lipid abnormality

2. Reduced HDL-Cholesterol: <40 mg/dl in males and < 50 mg/dl in females or history of specific treatment

3. Raised blood pressure: ≥130/85 mmHg or on treatment for previously diagnosed hypertension

4. Raised fasting blood sugar: ≥100 mg/dl or diagnosed type 2 diabetic mellitus

In this research, NCEP ATPIII criteria (Grundy et al., 2005) were adopted in this study due to laboratory limitations. Nevertheless, because all of the participants were from Thailand, anthropometric parameters (abdominal obesity) were use by WHO (2000) which defined obesity as waist circumference of ≥90 cm in men and ≥80 cm in women.

2.1.2 Current Treatment Protocols

Metabolic syndrome (MS) is a multifactorial disorder. As a result, the management approach is focused on the prevention and decrease of cardiovascular risk factors such as obesity, type 2 diabetes, and hypertension (Bianchi et al., 2007). Treatment goals are met through a variety of approaches, including lifestyle modification and pharmacological treatment (Grundy et al., 2005) as seen below.

2.1.2.1 Lifestyle Modifications (Lifestyle changes)

To lower the occurrence of MS, low carbohydrate diets, increased physical activity, and limited alcohol use are specifically targeted (Ferreira et al., 2005). Lifestyle adjustments have proven to be the most effective way to reduce the incidence of MS. A diet known as “the Mediterranean diet” is used to prevent MS (Galan-Lopez et al., 2019) This includes a variety of fruits and vegetables, whole grains, and healthy fats like seeds, nuts, and extra virgin olive oil. A modest number of fish, dairy, poultry, and red meat are also included (Davis et al., 2015). Research-based dietary guidelines

include 45% to 60% carbohydrate intake (mostly from legumes and whole grains), 10% to 20% protein, and 10% fatty acids (Mann, 2006).

Although frequent moderate physical activity improves MS, the highest outcomes are obtained when exercise is combined with an appropriate diet (Katzmarzyk et al., 2003). Anderssen et al. investigated the effects of combination interventions (physical exercise and limited diet) versus those without dietary restrictions. They showed a 67% and 24% reduction in MS incidence in the combined and isolated groups, respectively (Ekelund et al., 2007).

2.1.2.2 Pharmaceutical Management

Where alternative therapies have failed, pharmacological management can be tried. Strict glycemic and lipid profile control can reduce the risk factors for MS (Anioke et al., 2019). The following medication classes have been hypothesized to have a protective effect on the metabolic system (Elam et al., 2017). However, these results are debatable and require further investigation.

Metformin achieves the intended outcomes by several processes, including as decreasing glucose synthesis in the liver (Hundal et al., 2000), Increasing glucose reuptake by insulin receptor activation and activating tyrosine kinase in peripheral tissue (Gunton et al., 2003), reducing free fatty acid oxidation in adipose tissues (Riccio et al., 1996). Hundal et al. found that metformin's activities on hepatocytes caused a 25% to 30% drop in plasma glucose. Glitazones improve metabolic regulation by binding to PPAR-γ and moderating glucose metabolism. PPARs are ligand-inducible transcription factors that are part of the nuclear hormone receptor family. Three PPAR isoforms have been identified: PPAR-α, PPAR-β/δ, and PPAR-γ. These contribute to adipogenesis, inflammation, lipid and glucose metabolism (Botta et al., 2018).

Fenofibrate stimulates PPAR-α, while bezafibrate activates all three PPAR isoforms. A meta-analysis and found that fibrate medication significantly lowered fasting plasma glucose (-0.28 mmol/L), insulin levels (-3.87 pmol/L), and insulin resistance while having no effect on HbA1c (Panahi et al., 2018).

Statins are typically used to reduce LDL cholesterol in MS patients. Another essential role of statins known as pleiotropic effects is demonstrated to be reducing the risk of cardiovascular illnesses, which is significantly advantageous in reducing severe coronary events (Bianchi et al.,2007).

A result of the Pleiotropic effects: (Liao & Laufs, 2005)

1. Improved endothelial function
2. Inhibit the inflammatory process of blood vessels (Inflammatory Process)
3. Reduce the production of Fibrinogen
4. Increase the stability of platelets (Stabilize Platelet)

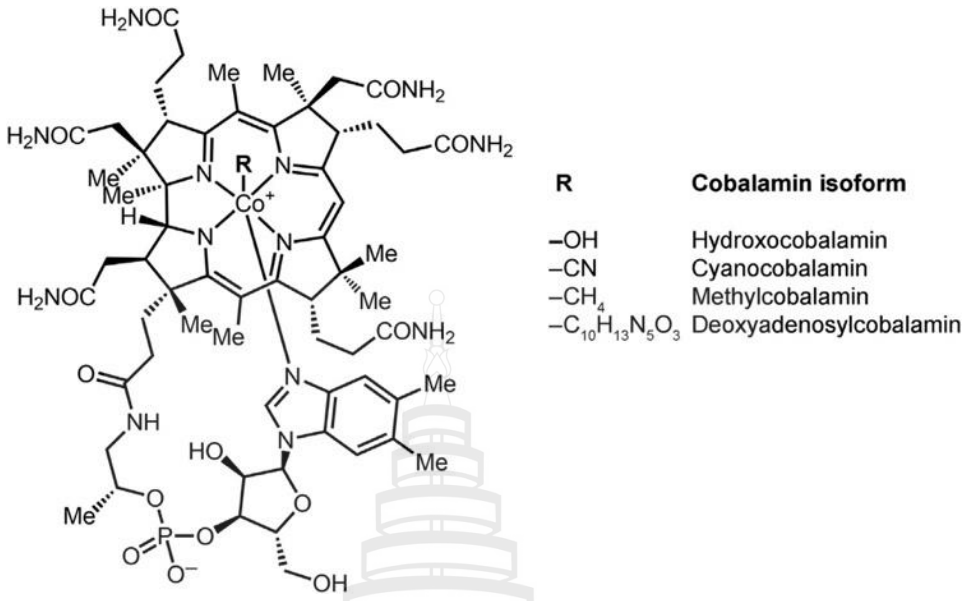
Moreover, MS has been linked to high C-reactive protein (CRP); statins lower CRP and diminish pro-inflammatory conditions (Grundy et al., 2005). Recent research has shown that combining statins with fibrates or nicotinic acid is more effective in reducing cardiovascular outcomes while also lowering the risk of myopathy.

2.2 Vitamin B12 (Cobalamin)

Vitamin B12 (cobalamin) is categorized as an essential water-soluble vitamin that is primarily derived from diet but is also synthesized naturally in humans by some large intestine-resident bacteria (Fang et al., 2017). Vitamin B12 was first isolated in the 1948.

2.2.1 Vitamin B12: Biochemical Structure

Vitamin B12 was structurally defined as a large organometallic complex whose size ranged between 1300 and 1500 Da. It consists of a central cobalt atom connected to six ligands, four of which are structurally reduced to create a corrin ring that joins and encircles the former by direct nitrogen connections. The α-axial 5,6-dimethylbenzimidazole (DMB) ligand below the central cobalt binds to the corrin ring through a phosphoribosyl moiety, providing excellent selectivity for intrinsic factor (IF) binding in the lower GI tract. The β-axial ligand (R-ligand) atop the corrin ring can take several forms, including methyl, 5’-deoxyadenosyl, hydroxo, acquo, or cyano. These are called methylcobalamin, deoxyadenosylcobalamin, hydroxycobalamin, acquocobalamin, and cyanocobalamin, respectively (Froese & Gravel, 2010).



Source Rizzo and Laganà (2020)

Figure 2.1 Cobalamin structure and isoforms

2.2.2 Vitamin B12: Nutritional Source

Natural sources are primarily found in animals and animal products. Excellent sources of vitamin B12 include meat, eggs, milk, dairy products, fish, and shellfish, with cobalamin concentrations ranging throughout ruminant-derived foods, with the highest amounts found in offal such as liver and kidney. Vitamin B12 is primarily lacking in plants; nevertheless, it is believed that traces of Vitamin B12 can be acquired in dried purple, green lavers, and some edible algae (Watanabe, 2007). Foods derived from plants that naturally contain high levels of cobalamin have been identified. Examples of these foods include dried shiitake mushroom fruiting bodies, dried green and purple laver (Enteromorpha and Porphyra species), aquatic plant Wolffia globosa (Mankai), granulate products and berries from sea buckthorn (Hippophae rhamnoides), and ground and dry extract from sidea couch grass (Elymus repens) (Sela et al., 2020).

Table 2.2 Variability of cobalamin (μg) concentration

	Cobalamin (μg)
Beef	
Brisket	2.25
Rip eye steak	1.73
Shoulder top blade steak	4.33
Sirloin cap steak	2.64
Tenderloin	3.47
Pork	
Leg (ham)	0.63
Shoulder	0.74
Sirloin	0.56
Spareribs	0.38
Tenderloin	0.52
Chicken	
Back	0.25
Breast	0.34
Drumstick	0.53
Leg	0.56
Thigh	0.62
Wing	0.25
Lamb	
Foreshank	2.34
Leg	2.50
Loin	2.04
Rip	2.09
Shoulder	2.53

Table 2.2 (continued)

	Cobalamin (µg)
Turkey	
Breast	0.42
Leg	0.39
Wing	0.39
Veal	
Leg (top round)	1.04
Loin	2.46
Rib	1.29
Shank	1.89
Shoulder	1.67
Sirloin	1.27

Note Variability of Cbl concentration in meat depends on species and cut. Selected cut (raw) from different species from US Department of Agriculture database are displayed.

Source Rizzo and Laganà (2020)

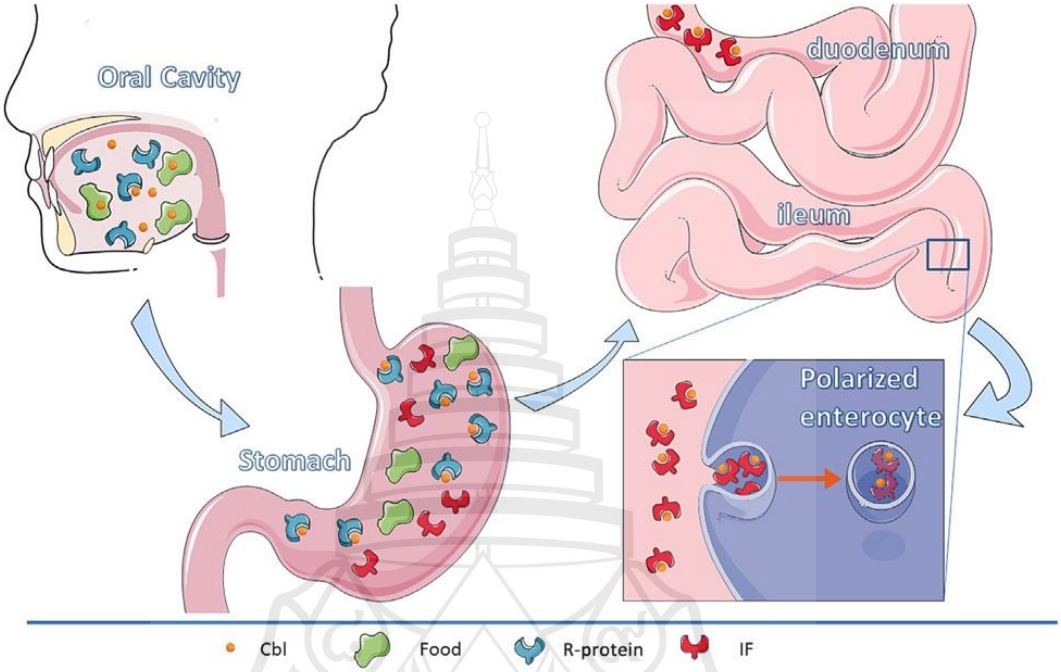
2.2.3 Vitamin B12: Absorption and Metabolism

Understanding absorption, metabolism, and the impact of changes in each of these processes is critical because it allows us to evaluate and predict pathological outcomes and therapeutic options.

2.2.3.1 Cobalamin (Cbl): Gastrointestinal absorption

The oral mucosa produces the first carrier R-protein (transcobalamin I). This molecule attaches to Cbl and then enters the stomach, where it dissociates. This protein shields Cbl from gastric acidity because its glycosylated structure is resistant to low pH (Hygum et al., 2011). This carrier has been discovered in many bodily fluids, including breast milk and plasma (Hvas et al., 2007). Pancreatic proteases enhance R-protein breakdown in the duodenum by releasing Cbl, which quickly binds to the second carrier

released by gastric parietal cells: intrinsic factor (IF) or Castle factor. Furthermore, this protein shields the vitamin from enzymatic digestion.



Source Rizzo and Laganà (2020)

Figure 2.2 Cobalamin gastrointestinal absorption

The gastric mucosa secretes intrinsic factor (IF), which is the primary regulator of absorption. IF attaches to cobalamin, generating a complex that is absorbed in the terminal ileum by Endocytic receptors and proteins responsible for vitamin B12 intestinal absorption, such as cubilin (CUBN), amnionless (AMN), receptor-associated protein (RAP), and megalin (LRP-2). This mechanism is responsible for absorbing at least 60% of oral coblamin.

2.2.3.2 Cobalamin (Cbl): Cellular uptake and Metabolism

The membrane megalin/transcobalamin II (TCII) receptor complex enables for cbl absorption in cells. The degradation of TCII and subsequent release of free cbl is required for vitamin B12 metabolic functions in the cytosol and mitochondria, which involve numerous processes and enzymes including methonine synthase (MS),

tetrahydrofolate (THF), methyltetrahydrofolate reductase (MTHFR), and methylmalonyl coA mutase (MCM).

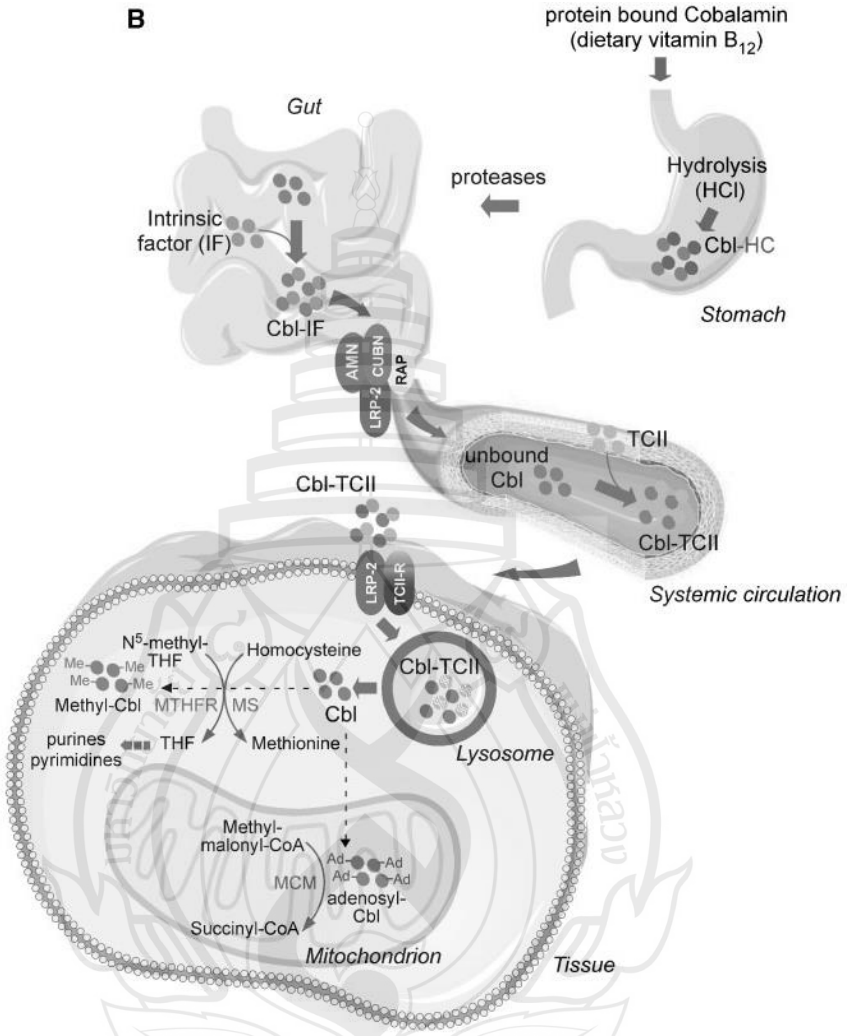
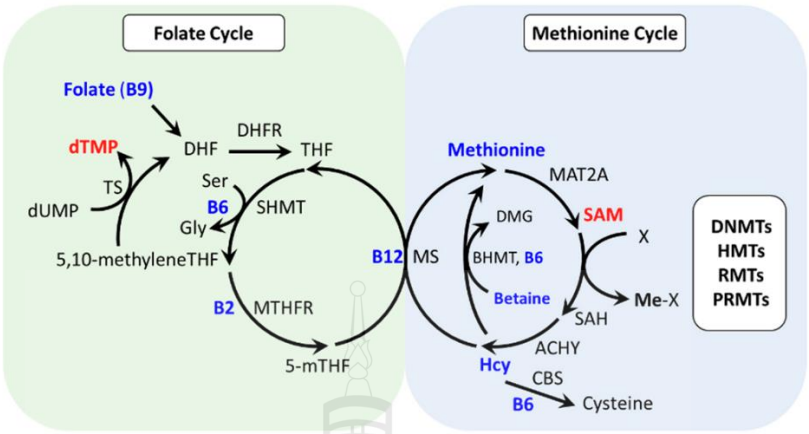


Figure 2.3 The metabolic journey of cbl from nutrient intake to its intestinal absorption

2.2.4 Vitamin B12: One-carbon Metabolism

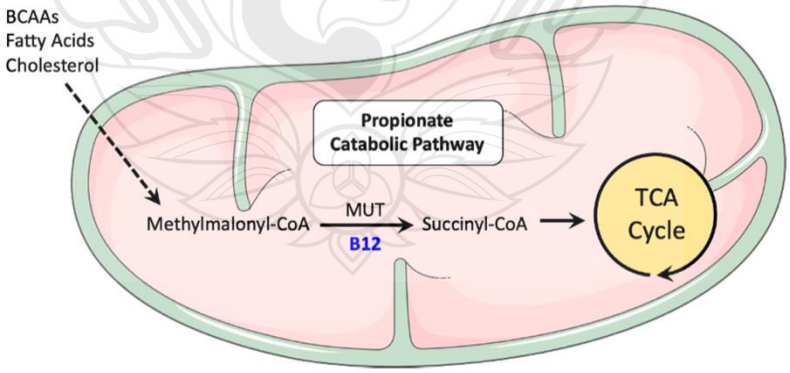
One-carbon metabolism, which controls DNA synthesis and methylation activities, is propelled by the folate and methionine cycles (Figure 2.4). Methionine synthase (MS), the rate-limiting enzyme in one-carbon metabolism, connects these two cycles by using 5-methyltetrahydrofolate (5-mTHF) as a methyl donor and vitamin B12 as a cofactor to convert homocysteine (Hcy) to methionine (Met).



Source Lyon et al. (2020)

Figure 2.4 One-carbon metabolism

Branching-chain amino acids (BCAAs), odd-chain fatty acids, and cholesterol are broken down via the propionate catabolic pathway so that they can be used by the mitochondria in the tricarboxylic acid cycle (TCA cycle). With adenosylcobalamin acting as a cofactor, methylmalonyl-CoA mutase (MUT) converts methylmalonyl-CoA to succinyl-CoA. The TCA cycle is then joined by succinyl-CoA.



Source Lyon et al. (2020)

Figure 2.5 Vitamin B12 and propionate metabolism

2.2.5 Vitamin B12: Deficiency

2.2.5.1 Dietary Reference Intakes (DRIs)

According to the European Society for Clinical Nutrition and Metabolism: ESPEN 2022, Dietary Reference Intakes (DRI) for healthy adults, based on maintenance of hematological status and serum cobalamin values is 2.4 mcg/day, 5 mcg/d in pregnancy and 4-5 mcg/d in lactation (Berger et al., 2022), while the American Society for Parenteral and Enteral Nutrition: ASPEN 2012 suggests current recommended adult daily oral micronutrient requirements as 2.4 mcg/day in healthy adults, 2.6 mcg/d in pregnancy and 2.8 mcg/d in lactation and parenteral micronutrient requirements is 5 mcg (Vanek et al., 2012).

Table 2.3 Recommended adult daily oral and parenteral micronutrient requirements according to American Society for Parenteral and Enteral Nutrition: ASPEN 2012

	Oral[1,a]	**Parenteral [23]**
Fat-Soluble vitamins		
Vitamin A	M, 900mcg or 3000 IU; F, 700 mcg or 2333 IU[b] 770 mcg (preg); 1300 mcg (lact)	990 mcg or 3300 IU[b]
Vitamin D	Age 19-70 y: 15 mcg or 600 IU[c, 35] Age >70y: 20 mcg or 600 IU[35]	5 mcg or 200 IU[c]
Vitamin E	15 mg; 19 mg (lact)	10 mg or 10 IU[d]
Vitamin K	M, 120 mcg; F, 90 mcg (AI)	150 mcg
Water-Soluble vitamins		
$Vitamin_1$ (thaiamine)	M, 1.2 mg; F, 1.1 mg 1.4 mg (preg/lact)	6 mg
$Vitamin_2$ (riboflavin)	M, 1.3 mg; F, 1.1 mg 1.4 mg (preg); 1.6 mg (lact)	3.6 mg
$Vitamin_3$ (niacin)	M, 16 mg; F, 14 mg 18 mg (preg); 17 mg (lact)	40 mg

Table 2.3 (continued)

	Oral[1,a]	**Parenteral [23]**
Vitamin$_5$ (panthotenic acid)	5 mg; 6 mg (preg); 7 mg (lact) (AI)	15mg
Vitamin$_6$ (pyridoxine)	Age 19-50 y: 1.3 mg Age >51 y: M, 1.7 mg; F, 1.5 mg 1.9 mg (preg); 2.0 mg (lact)	6 mg
Vitamin$_{12}$ (Cyanocobalamin)	2.4 mcg; 2.6 mcg (preg); 2.8 mcg (lact)	5 mcg
Vitamin C (ascorbic acid)	M, 90 mg; F, 75 mg 85 mg (preg); 120 mg (lact)	200 mg
Folate	400 mcg; 600 mcg (preg); 500 mcg (lact)	600 mg
Biotin	30 mcg; 35 mcg (lact) (AI)	60 mcg

2.2.5.2 Diagnostic Markers and Analytical Methods

Various studies have been conducted to measure the adequate dietary intake and normal range of vitamin B12 concentrations (Partearroyo et al., 2017). However, there was no international consensus on the lower and upper limit of vitamin B12 to define the deficiency. Most studies suggested that the accepted normal range should be between 197 and 771 pg/ml when using Electro-chemiluminescence immunoassay (ECLIA) method (Saraswathy et al., 2018; Gavars et al., 2022; Yılmaz & Yılmaz, 2016).

The deficiency of vitamin B12 also led to elevation of Homocysteine levels (Hcy) in blood circulation due to one-carbon metabolism. There was suggestion to apply serum homocysteine as biochemical markers for vitamin B12 status (McLean et al., 2008). Furthermore, serum Methylmalonic acid (MMA) was also sensitive and specific for vitamin B12 depletion which measured adenosyl-B12 function; however, some false MMA positives were seen in severe renal failure or major volume depletion (Vanek et al., 2012). It is worth mentioning that measuring serum methylmalonic acid

and homocysteine are specific and sensitive for screening and detecting vitamin B12 deficiency (Yamani et al., 2021).

2.2.5.3 Pathophysiology and Diseases

1. Nutrition

The risk of acquiring vitamin B12 insufficiency is higher in vegetarian populations, such as those in India (Saravanan & Yajnik, 2010). Cohort studies on vitamin B12 levels have found significant disparities between meat eaters, fish eaters, vegetarians, and vegans, with the results emphasizing the inadequate dietary intake among vegans and vegetarians (Sobiecki et al., 2016).

2. Surgical conditions

Many anatomical conditions have been linked to decreased absorption of vitamin B12, including Helicobacter pylori-induced stomach atrophy. A deficiency of intrinsic factors (IF) and gastric acid in the stomach causes pernicious anaemia. A bacterial overgrowth in the upper intestine prevents the absorption of vitamin B12 from food due to surgical conditions like low gastrointestinal motility brought on by gastrointestinal surgery, such as gastric bypass, jejunal diverticulosis, blind-loop syndrome following intestinal surgery, and structural abnormalities like diverticulosis in the duodenum or jejunum. The processes that have been proposed to explain this include hypochlorhydria, bacterial conversion of vitamin B12 to inactive analogues, and competition with gut microbes for the vitamin's uptake (Allen, 2008).

3. Infection

Human immunodeficiency virus, malaria, tapeworm, Giardia lamblia, and acquired immunodeficiency syndrome are diseases that obstruct the absorption of vitamin B12. Pathological diseases include tropical sprue, celiac disease, juvenile pernicious anemia, pancreatic insufficiency, and abnormalities in haptocorrin binding proteins are linked to decreased absorption of cobalamin. Trypsin synthesis is inhibited by cystic fibrosis and chronic pancreatitis, which is necessary to liberate vitamin B12 from transcobalamin-1 in the colon.

4. Inherited

Low serum cobalamin levels have been associated with congenital disorders, including transcobalamin I deficiency. a frequent gene variant in transcobalamin II, the vitamin B12 carrier protein, where proline (P) is substituted for

arginine (A), changing the mean total plasma concentrations of homocysteine and vitamin B12 (Seetharam et al., 1999).

5. Medication

Histamine receptor antagonists like famotidine, as well as proton-pump inhibitors like omeprazole and lansoprazole, can interfere with vitamin B12 absorption and raise the risk of insufficiency. Cimetidine inhibits the secretion of stomach acid, pepsin, and intrinsic factors (Steinberg et al.,1980).

6. Age

The prevalence of vitamin B12 deficiency is higher in patients over the age of 60 (Romain et al., 2016), which is similar to the European Society for Clinical Nutrition and Metabolism: ESPEN 2022 finding that adults aged 60 and over are at risk of vitamin B12 deficiency due to decreased absorption (Berger et al., 2022).

Table 2.4 Causes of vitamin B12 deficiency

Causes	**Examples**			
Lower intake of vitamin B12	1. Malnutrition	2. Vegetarian diet	3. Alcohol abuse	4. Old age (>60 years)
Damage to the Gastric Wall Impairing the Bioavailability of Vitamin B12 (with a decline in Intrinsic Factor)	1. Partial or total gastrectomy, including bariatric surgery, is an option for some stomach malignancies.	2. Atrophic autoimmune gastritis (such as pernicious anemia) or other gastritis (e.g., Helicobactor pylori).		
Deterioration of Absorption Through the Intestines	1. Blind-loop syndrome	2. Infections with tapeworms, giardiasis, and bacterial overgrowth	3. Ileal resection	4. Crohn’s disease
Vitamin B12 Deficiency Disorders Inherited (Congenital)	1. Intrinsic factor receptor malfunction, as shown in Imerslund-Gräsback syndrome	2. Juvenile pernicious anemia, Congenital intrinsic factor (IF) deficiency	3. Cobalamin mutation	4. Deficiency in Transcobalamin (TC)

Table 2.4 (continued)

Causes	Examples		
Higher Needs for Vitamin B12	1. Hemolytic anemic conditions	2. HIV infection	
Medication	1. Metformin	2. Proton pump inhibitors	3. Prolonged use of histamine receptor 2 (H2) blockers (especially >12 months)

Source Hunt et al. (2014)

2.2.5.4 Vitamin B12 level-symptoms correlation

Vitamin B12 deficiency is associated with varied multi-systemic manifestations, mainly causes a wide series of neurological, gastrointestinal, and hematological symptoms. Axonal demyelination and degeneration are the classic signs of neuronal damage caused by a vitamin B12 shortage, presenting as severe peripheral or autonomic neuropathy, spinal cord degeneration, psychosis, and dementia (Davis et al., 2013). Common neurological features are paresthesia, peripheral neuropathy, myelopathy, neuropsychiatric manifestation, cognitive impairment (Sahu et al., 2021). Vitamin B12 levels are also related to several mental health disorders, such as anxiety, schizophrenia, obsessive compulsive disorder as vitamin B12 is essential in the synthesis of neurotransmitters including dopamine and serotonin (Tan et al., 2023). Screening for vitamin B12 deficiency might intervene against any potential complications, it has been shown that early detection of the condition provides good prevention against the development of a potentially disabling, painful, and irreversible nerve injury (Fasipe et al., 2020).

The major non-neurological symptoms are atrophic glossitis, stomatitis, mucositis, gastritis, and malabsorption from defective cell repair processes. Vitamin B12 deficiency is associated with hematological findings such as macrocytic red blood cells, hypersegmented white blood cells and pancytopenia. Frequent presenting signs

are pallor, hyperpigmentation of skin and knuckles, edema, icterus or jaundice, splenomegaly, and hepatomegaly (Davis et al., 2013).

2.3 Relationships between Metabolic Syndrome and Vitamin B12

Numerous research have been conducted globally on the connection between vitamin B complex, particularly vitamin B12, and metabolic syndrome. Numerous research studies have demonstrated a favourable correlation between vitamin B12 supplements and their efficacy in mitigating cardiovascular disease (Kataria et al., 2021) and insulin resistance (Satapathy et al., 2020). Some studies found a negative or inverse association between serum vitamin B12 concentrations to Obesity (Sun et al., 2019). Therefore, the relevant papers involved in our research were categorized into pre-clinical and clinical studies as seen below.

2.3.1 Evidence from Pre-clinical Studies

2.3.1.1 Non-human studies

According to a study conducted on mouse models with severe and moderate vitamin B12 insufficiency, the plasma cholesterol, triglycerides, and body fat percentage of the severely deficient mice were considerably greater than those of the moderately deficient animals and the control group. Severely deficient animals also showed markedly elevated levels of plasma homocysteine, plasma leptin, and IL-6, as well as elevated oxidative stress (measured by MDA and protein carbonyls) and decreased antioxidant defense (measured by SOD and catalase). According to this study, a severe vitamin B12 shortage from an early age likely throws off the metabolic pathways, which causes obesity, which is recognized as a chronic low-grade inflammatory disease. The findings indicate that proinflammatory adipocytokines, like TNF-α and IL-6, are markedly elevated in vitamin B12 deficient individuals. This could trigger the hypothalamic-pituitary-adrenocortical (HPA) axis, releasing cortisol and causing oxidative stress accumulation, both of which have been connected to obesity in human and rodent models (Ghosh et al., 2016).

2.3.1.2 In Vitro studies

In recent study, the cellular mechanism in human preadipocyte cell line induced by low vitamin B12 involved the elevated levels of cholesterol and TG compared with controls (Adaikalakoteswari et al., 2017). This was explained by vitamin B12-deficient conditions increased the gene expression of key transcriptional regulators of adipogenic differentiation such as peroxisome proliferator–activated receptor g (PPARg) and CCAAT/enhancer–binding protein a (CEBPa). These results imply that adipocytes with low vitamin B12 levels may be induced to undergo lipogenesis and adipogenesis. Low vitamin B12 increased adipogenesis (PPARg, CEBPa, RXRa) and lipogenesis (FASN, ACACA) in vitro (adipocytes) and in vivo (adipose tissue during childbirth). These results show that low B12 affects the two different processes of adipose tissue development. Additionally, low B12 caused hypomethylation of the cholesterol transcription factor SREBF1, which is known to induce PPARg and regulate genes required for lipogenesis. Since vitamin B12 is a crucial micronutrient required for the majority of tissues to function, hepatocytes may likewise go through comparable processes. Higher circulating lipid levels could potentially result from hepatocytes experiencing such dysregulation of lipids. Through a variety of processes, such as DNA methylation, histone modification, chromatin remodeling, and variations in the expression of small and long noncoding RNAs like miRs (microRNA), nutrient imbalances can result in epigenetic modifications. It was demonstrated by in vitro adipocyte research that these miRs were markedly changed in vitamin B12-deficient environments. Transcirculating miRs offer a potential pathway for inter-tissue communication. These aberrant miRs have the potential to induce harmful epigenetic modifications in the tissues of the kids and predispose them to metabolic diseases in later life if they are carried across the placenta. Further research is required to validate these conjectures.

Another study of adipocyte cell cultures from women of non-pregnant and childbearing age (in early pregnancy and at delivery) found that vitamin B12 deficiency is an important metabolic risk factor because vitamin B12 regulates S-Adenosyl-l-Methionine (AdoMet) to S-adenosyl-L-homocysteine (AdoHcy) levels, which influences the cholesterol biosynthesis pathway in human adipocytes. Their findings demonstrate that vitamin B12 plays an important role in cholesterol biosynthesis by

inducing SREBP gene expression in human adipocytes and DNA hypomethylation. In vitamin B12-deficient conditions, protein, and mRNA expressions of both SREBPs were significantly increased. This study also discovered that the other gene expressions of the cholesterol biosynthesis gene (HMGCR) and cholesterol regulatory gene (LDLR) were considerably elevated in the low vitamin B12 group compared to the control, supporting the role of vitamin B12 in cholesterol biosynthesis in vivo (Adaikalakoteswari et al., 2017).

Table 2.5 The effect of low vitamin B12 levels on metabolic risk in pre-clinical studies

Obesity	**Insulin Resistance**	**Dyslipidemia**	**Cardiovascular Diseases**
Wistar rats had higher levels of visceral obesity. (Kumar et al., 2013)	Elevated blood pressure and increased insulin resistance in sheep. (Sinclair et al., 2007)	Higher levels of TG, total cholesterol, and obesity in Wistar rat models. (Kumar et al., 2013)	Low B12 causes disruption of androgen testosterone levels in Sprague-Dawley rat model, which is linked to vascular dysfunction. (Lodhi & Panchal, 2014)
Female C57 BL/6 mice have higher levels of obesity. (Ghosh et al., 2016)		Elevated plasma TG, cholesterol, and some pro-inflammatory indicators. (Ghosh et al., 2017)	In rat models, B12 and/or folate administration reduced the incidence of myocardial infarction (MI) and tHcy levels. (Hagar, 2002)
Wistar rats' increased body weight. (Kumar et al., 2013)		Increased cholesterol levels in the human adipocyte cell line (Chub-S7). (Adaikalakoteswari et al., 2017)	

Table 2.5 (continued)

Obesity	Insulin Resistance	Dyslipidemia	Cardiovascular Diseases
Wistar rats had greater overall body fat. (Ghosh et al., 2017)		Increased TG in the human adipocyte cell line (Chub-S7). (Adaikalakoteswari et al., 2017)	

Source Boachie et al. (2020)

2.3.2 Evidence from Clinical Studies

According to the body mass index-standard deviation score quartiles, the children were divided into three groups for the purpose of a study on the relationship between vitamin B12 status, the severity of obesity, and metabolic syndrome in obese children. Group 1 included the first quartile, group 2 included the second and third quartiles, and group 3 included the fourth quartile. The study found that mean vitamin B12 levels were significantly lower in obese children compared to healthy volunteers ($p<0.001$) as well as lower in obese children with MS (metabolic syndrome) than in those without MS. Additionally, the study discovered that a higher body mass index standard deviation score (BMI-SD) was adversely correlated with the degree of obesity and linked to a drop in vitamin B12 levels (Özer et al., 2017).

An Indian study on vitamin B12 levels in mothers discovered that low levels of the vitamin in the mother raised the chance that the offspring will grow up to be more obese and insulin resistant. Research by Yajnik et al. revealed that vitamin B12 deficiency is frequent during pregnancy, contributes to hyperhomocysteinaemia, and predicts increased insulin resistance. The study examined the relationship between the mother's vitamin B12, folate, and tHcy status during pregnancy and the impacts of the offspring. The findings revealed that 30% of pregnant women had increased tHcy concentrations (>10 μmol/l), 90% had elevated MMA (>0.26 μmol/l), and the majority (>60%) had low plasma vitamin B12 concentrations (<150 pmol/l). More adipose

(higher body fat percentage and higher central fat) but smaller newborn size were predicted by higher maternal tHcy concentrations, which were linked to low vitamin B12 concentrations. This is because a vitamin B12 deficiency will trap folate as 5-methyltetrahydrofolate, prevent the generation of methionine from homocysteine, and therefore reduce protein synthesis and lean tissue deposition. Increased lipogenesis may be caused by high methylmalonyl-CoA because it inhibits carnitine palmitoyltransferase, which in turn inhibits β-oxidation in lipid metabolism. The findings of this study indicated that intrauterine programming may have a significant role in the development of adult metabolic illnesses by causing abnormalities in one-carbon metabolism caused by low vitamin B12 concentrations (Yajnik et al., 2008).

Saraswathy et al. (2018) investigated the correlations between homocysteine, dyslipidemia, and obesity parameters (WC, BMI, WHR) and vitamin B12 and folate deficiencies. The obesity characteristics were assessed in healthy individuals without a metabolic problem. An IV blood sample was obtained to measure the levels of homocysteine, folate, vitamin B12, and lipid profile. The findings demonstrated a substantial correlation between low HDL and hyper-homocysteinemia and vitamin B12 insufficiency. Vitamin B12 deficiency was brought on by low HDL (dyslipidemia), which also caused metabolic disruption in the lipid and one-carbon metabolism pathways. This is because vitamin B12 deficiency may have prevented methylmalonyl CoA (MM-CoA) from being converted to succinyl-CoA, which led to an accumulation of MM-CoA. This accumulation further inhibited the activity of the fatty acid oxidation enzyme, carnitine palmitoyl transferase (CPT1), which in turn caused lipogenesis. Furthermore, this study revealed that a lack of vitamin B12 could impair the function of the methionine synthase (MS) enzyme, which is essential for the conversion of homocysteine to methionine, resulting in an increase of homocysteine levels. In conclusion, this study revealed that low HDL initiated vitamin B12 insufficiency, which is the main culprit, disrupting two metabolic pathways and generating hyperhomocysteinemia, which is at risk for cardiovascular adversity (Saraswathy et al., 2018).

The three patient groups (T2DM, CVD, and hypertension) all had elevated levels of vitamin D2 and homocysteine, while folic acid and vitamins D3 and B12 were below the reference range, according to a study on the relationships between the serum

levels of these substances in patients with metabolic diseases. The lowest levels of homocysteine were correlated with the highest levels of folic acid, vitamins D2 and D3, and all patient groups; however, the lowest levels of homocysteine were only correlated with the highest levels of vitamin B12 in patients with CVD ($P < 0.05$), suggesting that the benefits of vitamin B12 supplements are limited to lowering the risk of CVD. According to this study, deficiencies in the vitamins D and B12 may lead to higher levels of homocysteine in the serum by preventing its conversion to methionine during one-carbon metabolism. In the trans-sulfuration and amino acid methylation processes, homocysteine plays a crucial role as an intermediary. These pathways depend on folic acid and vitamin B12 as essential coenzymes. Elevated serum homocysteine has been directly linked to metabolic illnesses, including cardiovascular disease (CVD), hypertension, type 2 diabetes (T2DM), and problems connected to T2DM. This is because homocysteine can directly damage blood vessels (Mao et al., 2016).

Another study looks at the relationships between the concentrations of biomarkers involved in one-carbon (1-C) metabolism, such as total homocysteine (tHcy), folate, and vitamin B12, and the mortality rates from cardiovascular disease (CVD) and other causes among the very old (older than 85). After adjusting for important sociodemographic, lifestyle, and health variables, the results, which were expressed as hazard ratios (HR), showed that women with higher plasma vitamin B12 concentrations had an increased risk of cardiovascular and all-cause mortality as well as a higher risk of death from cardiovascular disease than did those with lower concentrations of tHcy. According to the study, high homocysteine levels may raise the risk of cardiovascular disease by promoting endothelial dysfunction, atherothrombosis, and lipid peroxidation. Methionine synthase (MS), which transmethylates homocysteine to methionine and produces S-adenosylmethionine (SAM), and methylmalonyl-CoA mutase, which changes methylamalonyl-CoA into succinyl-CoA in the fatty acid synthesis pathway, both rely on vitamin B12 as a cofactor. SAM is essential for controlling gene expression since it is the universal methyl donor for methylation of all biological macromolecules, including DNA and histones. considering that 1-C metabolism depends on it. Thus, homocysteine, folate, and vitamin B12 have been linked to cardiovascular disease (CVD) and all-cause mortality, according to this study's conclusion (Mendonça et al., 2018).

According to a systematic review, there is a wealth of literature regarding the relationship between homocysteinemia and vitamin B12 deficiency, as well as the latter's function in the development of atherosclerosis and other forms of coronary artery disease (CAD). Due to their close relationship to the metabolism of homocysteine and methionine, folate, and vitamin B12 (cyanocobalamin) deficiencies, as well as hyperhomocysteinemia, have been shown in numerous studies to be risk factors for cardiovascular disease. Vitamin B12 is a component of the folate-independent metabolic pathway in the mitochondria and aids in the control of the metabolism of methylmalonic acid (MMA). Elevated MMA levels in most patients' serum levels indicated a symptomatic vitamin B12 insufficiency. The examination into the association between CVD and vitamin B12 was limited, but meta-analyses involving prospective studies have repeatedly demonstrated correlations with tHcy and increased risk of CVD. It was challenging to ascertain the specific impact of vitamin B12 in several randomized controlled trials (RCTs) utilizing vitamin B12 compensation (varying from 6 μg-1 mg) in conjunction with folate. Lower amounts of vitamins B have been linked to a higher risk of stroke; supplementing with dosages of vitamin B12 ranging from 0.02-1 mg/d results in a 7% decrease in tHcy, while folate provides a 10% to 30% decrease in CVD risk. In individuals with sufficient folate status, vitamin B12 has also been demonstrated to be a significant predictor of tHcy concentrations (Chakraverty & Chakraborty, 2018).

Table 2.6 The effect of low vitamin B12 levels on metabolic risk in clinical studies

Obesity	Insulin Resistance	Dyslipidemia	Cardiovascular Diseases
Low B12 (<150 pmol/L) was linked to higher T2D risk and increased adiposity in pregnant women (Yajnik et al., 2008).	B12 levels below 180 pmol/L were linked to higher insulin resistance in pregnant White Caucasian women without gestational diabetes mellitus (Knight et al., 2015).	Elevated levels of LDL, total cholesterol, and the cholesterol-to-HDL ratio were linked to low B12 levels (<148 pmol/L) in women of reproductive age, both pregnant and non-pregnant. (Adaikalakoteswari et al., 2015).	Total homocysteine (tHcy) levels and B12 were adversely linked in Chinese patients with CVD who were 65 years of age or older (median low B12 = 4.19 pmol/L). (Mao et al., 2016)

Table 2.6 (continued)

Obesity	Insulin Resistance	Dyslipidemia	Cardiovascular Diseases
Elevated BMI was linked to low B12 (median −203 pmol/L and <148 pmol/L) in maternal, general, and/or clinical groups, respectively (Wiebe et al., 2018).	Prediction of increased risk of insulin resistance in offspring born to moms with low B12 levels (<150 pmol/L, 148 pmol/L) (Stewart, et al., 2011).	In the North Indian population, low B12 (≤220 pg/mL) was linked to both low HDL and hyperhomocysteinemia. (Saraswathy et al., 2018).	In older women, low B12 (<148 pmol/L) and high total homocysteine (tHcy) levels were linked to an increased risk of mortality from CVD and all causes (Mendonça et al., 2018).
Low B12 (102-208 pmol/L interquartile range) was related with obesity in children as compared to healthy volunteers (Özer et al., 2017).	Reduced levels of B12 (≤150 pmol/L, <148 pmol/L, <200 pg/mL, <178 pg/mL) have been linked to higher levels of insulin resistance in adult patients, women with polycystic ovarian syndrome, and obese teenagers (Ho et al., 2014).	Low HDL and high TG levels in erythroid individuals are examples of MS indicators that have a negative correlation with B12 (low B12 range 180–301 pmol/L). (Guarnizo-Poma et al., 2018).	When B12 was taken supplements, patients with CVD and/or renal disease had a lower risk of stroke. (Chakraverty & Chakraborty et al.,2018).

Source Boachie et al. (2020)

CHAPTER 3

RESEARCH METHODOLOGY

The study aimed to evaluate serum vitamin B12 concentrations across people with and without metabolic syndrome. All protocols and techniques used in this study were reviewed and approved by the faculty of anti-aging and regenerative medicine at Mae Fah Luang University in Thailand.

3.1 Study Design

A cross-sectional study.

3.2 Population and Sample Size

3.2.1 Population

Participants with age more than 45 years old including adults with metabolic syndrome and without metabolic syndrome, most participants were nursing college staffs from Boromarajonani College of Nursing, Nonthaburi, Thailand who were willing to participate in February 2024. All patients provided written informed permission before to their participation.

3.2.2 Sample

Total participants were recruited initially then separated into 2 groups as patients with metabolic syndrome and non-metabolic syndrome.

3.2.3 Sample Size Determination

The calculation as following defined an adequate sample size.;

$$n = \frac{Z_{\alpha/2}{}^2 \sigma^2}{d^2}$$

n = Sample size

$Z_{\alpha/2}$ = Standard value under normal distribution at Confident Interval 95% (1.96)

σ^2 = Population variance of serum vitamin B12 concentrations (1.69)

d^2 = The degree of maximum error allowed for estimate σ^2 (0.55)

σ referred from "Elevated Total Homocysteine in All Participants and Plasma Vitamin B12 Concentrations in Women Are Associated with All-Cause and Cardiovascular Mortality in the Very Old: The Newcastle 85+ Study" (Mendonça et al., 2018).

Calculating $n = \frac{(1.96)^2(1.69)}{(0.55)^2}$

n = 21.46

n = ~ 21

n = 21, calculate 20 % contingency = 4.2 ~ 4

sample size per group = 21 + 4 = 25 subjects

Total sample size was 50 subjects: 25 subjects per group.

3.3 Selection Criteria

3.3.1 Inclusion Criteria

3.3.1.1 Thai participants aged more than 45 years old.

3.3.1.2 Participants with and without metabolic syndrome. According to NCEP ATP III criteria and WHO classification for asian, participants with metabolic syndrome reach more than three of five criteria:

1. High fasting blood sugar: ≥100 mg/dL
2. Hypertriglyceridemia: ≥150 mg/dL
3. Low High-Density Lipoprotein Cholesterol: <40 mg/dL in men and <50 mg/dL in women
4. High blood pressure: >130/85 mmHg
5. Abdominal obesity: WC ≥90 cm in men and ≥80 cm in women

(participants who do not meet more than three of five criteria listed above are considered without MS)

3.3.1.3 Participants accepted to examine blood samples.

3.3.1.4 Participants willing to participate in the study.

3.3.2 Exclusion Criteria

3.3.2.1 Participants with a vitamin B supplement or medical treatment involved vitamin B within 12 months prior to study.

3.3.2.2 Participants with any medical or surgical conditions which could reduced vitamin B12 intake or absorption.

3.3.2.3 Participants with any anemic diseases such as pernicious anemia, hemolytic anemia or anemia of chronic disease.

3.3.2.4 Participants with vegetarian/vegan dietary, under malnutrition, or have a history of chronic alcohol consumption.

3.3.2.5 Participants with a history of autoimmune disease, malignancy, chronic infection, or chronic liver or kidney disease.

3.3.3 Withdrawal and Discontinuation Criteria

3.3.3.1 Participants who are not consent for participating in the study

3.3.3.2 Participants who cannot take blood samples

3.3.3.3 Other medical conditions

3.3.4 Study Location

Boromarajonani College of Nursing, Nonthaburi, Thailand

3.4 Research Tools and Equipment

3.4.1 Anthropometric Measurements (Body Composition)

3.4.1.1 Weight (kg), Height (cm) and BMI (kg/m^2)

were measured using a digital scale for weight and a portable stadiometer for height while subjects were barefoot and wearing light clothing.

3.4.1.2 Waist circumference: WC (cm), Hip circumference: HC (cm) were measured.

3.4.1.3 Blood pressure (mmHg)

was measured using a digital sphygmomanometer with an appropriate collar.

3.4.2 Biochemical Data (Laboratory)

by Bangkok Medical Laboratory (BML)

3.4.2.1 Blood for metabolic diseases

1. Serum fasting glucose (FBS): Enzymatic hexokinase method
2. Total cholesterol (TC): Enzymatic colorimetric method
3. Triglycerides (TG): Enzymatic colorimetric method
4. LDL-cholesterol: Homogeneous enzymatic colorimetric assay
5. HDL-cholesterol: Homogeneous enzymatic colorimetric assay

3.4.2.2 Serum vitamin B12 concentrations

using Electrochemiluminescence immunoassay (ECLIA)

3.4.3 Other General Data and Confounders

Age, gender, occupation, physical activity, smoking, and alcohol consumption were collected using questionnaires. All these data were used for checking confounders.

3.5 Research Methods

In February 2024, this study was carried out at Boromarajonani College of Nursing in Nonthaburi, Thailand.

Methods:

1. Enrolling participants.
2. Describe the study's methodologies and objectives.
3. Participants signed the informed consent, written informed consent was obtained before their participation in our study.
4. The subject’s information was collected using questionnaires (general data, underlying diseases, current medications, and other confounders).

5. Subjects were measured the body composition (anthropometric measurements) including weight (kg), height (centimeter), BMI (kg/m^2), waist circumference (centimeter), hip circumference (centimeter), waist hip ratio and blood pressure (mmHg).

6. Intravenous blood sample (20 mL) was collected by trained medical staffs, stored at room temperature, and were obtained after a 10- to 12-hour fast.

7. Data is collected, analyzed, discussed, and concluded.

3.6 Data Collecting, Outcome Measurement and Cut-offs

3.6.1 Cut-offs for Anthropometric Parameters (Body Composition)

3.6.1.1 Weight (kg), Height (cm) and BMI (kg/m^2)

According to WHO (2000), obesity was defined as below.

Table 3.1 Classification by BMI in adults Asians

Classification	BMI (kg/m^2)
Underweight	< 18.5
Normal range	18.5-22.9
Overweight	≥ 23
At risk	23-24.9
Obese I	25-29.9
Obese II	≥ 30

Source WHO (2000)

3.6.1.2 Waist circumference: WC (cm), Hip circumference: HC (cm) and Waist hip ratio

According to WHO (2000),

Obesity was defined as WC ≥ 90 cm for men

WC ≥ 80 cm for women

High WHR was defined as > 0.90 for men

> 0.80 for women

3.6.2 Cut-offs for Metabolic Syndrome Including Blood Pressure and Biochemical Parameters (Laboratory), According to NCEP ATPIII Criteria

3.6.2.1 Blood pressure (mmHg)

According to NCEP ATP III criteria (Grundy et al., 2005)

Hypertensive considered as blood pressure ≥ 135/85 mmHg

3.6.2.2 Serum fasting glucose (FBS)

Table 3.2 NCEP ATP III criteria for fasting sugar levels 2005

Serum fasting glucose	Values (mg/dL)
hypoglycemia	< 70
normal	70 - 100
prediabetes	101 - 125
diabetes	≥ 126

3.6.2.3 Dyslipidemia (DLP)

1. Total cholesterol (TC)
2. Triglycerides (TG)
3. Low-density lipoprotein cholesterol (LDL-C)
4. High-density lipoprotein cholesterol (HDL-C)

Table 3.3 NCEP ATP III criteria for dyslipidemia 2005

Dyslipidemia	Values (mg/dL)
high Total cholesterol	≥ 200
high Triglycerides	≥ 150

Table 3.3 (continued)

Dyslipidemia	Values (mg/dL)
low HDL-Cholesterol	< 40(M), < 50(F)
high LDL-Cholesterol	≥ 130

3.6.3 Cut-offs Used for Serum Vitamin B12 Concentrations (Laboratory)

Serum vitamin B12 concentrations by Electrochemiluminescence immunoassay (ECLIA) method (Saraswathy et al., 2018; Gavars et al., 2022; Yılmaz & Yılmaz, 2016; Sukla & Raman, 2011) and Bangkok Medical Laboratory (BML)

Table 3.4 Serum vitamin B12 concentrations range

Serum vitamin B12 concentrations	Values (pg/ml)
Accepted normal range	197-771

3.6.4 Other General Data and Confounders

Age, smoking, alcohol consumption, physical activity and occupation were collected using questionnaires. All these data were used for checking confounders.

3.6.4.1 Age were categolized in two groups: 45-59 years old and ≥60 years old, according to the European Society for Clinical Nutrition and Metabolism: ESPEN, 2022 and the study by M. Romain, finding that adults aged 60 and over are at risk of vitamin B12 deficiency due to decreased absorption (Berger et al., 2022; Romain et al.,2016).

3.6.4.2 Smoking was classified as individuals with current smoker who has smoked 100 cigarettes in his or her lifetime and who currently smokes cigarettes, according to CDC definition (National Center for Health Statistics, 2018).

3.6.4.3 Alcohol drinking was classified depend on gender, according to CDC definition (National Center for Health Statistics, 2018)

1. For females: Having more than 3 drinks but no more than 7 drinks per week, on average over the past year.

2. For males: Having more than 3 drinks but no more than 14 drinks per week for men, on average over the past year

3.6.4.4 Physical activity was self-reported as active or sedentary; routine walking, standing, or physically intensive work were categorized as active participants, while those without an active habit were labelled as sedentary participants.

3.6.4.5 Occupation was majorly categorized as agricultural workers, government employees, company employees, self-employed, business owners, students retired persons, or others

3.7 Data Analysis and Statistics

The medical record data and outcomes of the participants in this research were recorded by Microsoft 365 Excel 2021.

Statistical analysis

1. Statistical analysis was performed using SPSS software version 26.0 (IBM).

2. The continuous data are presented as means ± standard deviation (SD).

3. Outliers were checked for all variables using histogram and Box plot and were removed.

4. Normality test was performed by Shapiro-Wilk test and Kolmogorov–Smirnov test.

5. Independent sample t-test was used to compare between normal distribution data (parametric test) while in skewed variables, Mann-Whitney U-test was performed (non-parametric test).

6. A significance level of 95% was used for all the statistical tests. $P<0.05$ was considered significant.

3.8 Ethical Consideration

All protocols and techniques utilized in this study were evaluated and approved by the Faculty of Anti-aging and Regenerative Medicine at Mae Fah Luang University in Thailand. All participants were told about all aspects of the study and completed an informed consent form. Participants might stop the trial at any moment with no penalty. All of the data collected during the study were anonymous and kept totally confidential.

CHAPTER 4

RESULTS

4.1 The General Data Analysis

Table 4.1 General characteristic profiles between groups

Characteristics	Metabolic syndrome† (n=26)		Non-metabolic syndrome‡ (n=26)	
	Number	**Percent (%)**	**Number**	**Percent (%)**
Sex				
Male	8	30.77	2	7.69
Female	18	69.23	24	92.31
Age				
45-59	18	69.23	22	84.62
≥ 60	8	30.77	4	15.38
Physical activity				
Active	16	61.54	21	80.77
Sedentary	10	38.46	5	19.23
Smoking				
Yes	4	15.38	2	7.69
No	22	84.62	24	92.31
Alcohol drinking				
Yes	5	19.23	0	0.00
No	21	80.77	26	100.00

There were 52 subjects participated in this study, divided into metabolic syndrome group consisted of 26 subjects and non-metabolic syndrome group consisted of 26 subjects. The descriptive statistics was used to determine the demographic profile of subjects categorized into sex, age, physical activity, smoking and drinking behavior, as shown in Table 4.1

–† participants with metabolic syndrome reach more than three of five criteria:

1. High fasting blood sugar: ≥100 mg/dL
2. Hypertriglyceridemia: ≥150 mg/dL
3. Low High-Density Lipoprotein Cholesterol: <40 mg/dL in men and <50 mg/dL in women
4. High blood pressure: >130/85 mmHg
5. Abdominal obesity: WC ≥90 cm in men and ≥80 cm in women

–‡ participants who do not meet more than three of five criteria listed above are considered without metabolic syndrome (According to NCEP ATP III criteria and WHO classification for asian)

Table 4.2 General parameters including anthropometric and biochemical parameters between metabolic syndrome and non-metabolic syndrome group

Parameters	**Metabolic syndrome (n=26)**	**Non-metabolic syndrome (n=26)**	**p-value**
	(mean ± SD)	**(mean ± SD)**	
Weight (kg)	76.58 ± 17.11	56.62 ± 9.22	<0.001^a
Male	89.38 ± 14.97	67.5 ± 17.68	0.109^a
Female	70.89 ± 15.06	55.71 ± 8.23	<0.001^a
Height (cm)	162.19 ± 8.66	157.62 ± 5.90	0.030^a
Male	171.88 ± 6.36	165 ± 0	0.018^a
Female	157.89 ± 5.48	157 ± 5.72	0.615^a
BMI (kg/m^2)	29.01 ± 5.64	22.81 ± 3.70	<0.001^b
Underweight (<18.5)	N/A	18.11 ± 0.24	-

Table 4.2 (continued)

Parameters	Metabolic syndrome (n=26) (mean ± SD)	Non-metabolic syndrome (n=26) (mean ± SD)	p-value
Normal (18.5-22.9)	N/A	20.63 ± 1.29	-
Overweight (23-24.9)	24.22 ± 0.62	23.66 ± 0.76[§]	0.190[a]
Obese I (25-29.9)	27.03 ± 1.39	26.84 ± 1.48[§]	0.807[a]
Obese II (≥30)	35.45 ± 4.53	N/A	-
WC (cm)	98.48 ± 14.26	78.33 ± 12.03	<0.001[a]
HC (cm)	108.79 ± 14.81	94.13 ± 9.37	<0.001[a]
FBS (mg/dL)	119.04 ± 29.95	88.04 ± 6.58	<0.001[b]
T.chol (mg/dL)	200.77 ± 52.59	204.85 ± 41.93	0.759[b]
TG (mg/dL)	155.31 ± 73.11	85.42 ± 34.14	<0.001[a]
HDL (mg/dL)	54.81 ± 12.22	71.31 ± 17.09	<0.001[a]
LDL (mg/dL)	114.46 ± 44.17	119.12 ± 32.49	0.667[b]
Vitamin B12 (pg/ml)	693.02 ± 210.57	634.75 ± 345.18	0.182[b]

Note N/A = Unable to analyse due to the group contains information that is less than or equal to one.

The table represents data of weight, height, body mass index (BMI), waist circumference (WC), hip circumference (HC), fasting blood sugar (FBS), lipid profiles (T.chol, TG, HDL, LDL) and serum vitamin B12 level of the subjects. There were significant differences in weight, height, BMI, WC, HC, FBS, TG, HDL between two groups while using statistical analysis by independent t-test^{a} and Mann–Whitney U test^{b} ($p<0.05$).

Participants with BMI in overweight and obese I category but do not meet the metabolic syndrome criteria are classified as non-metabolic syndrome[§]

4.2 Comparisons of Serum Vitamin B12 Concentration and other Parameters

Comparisons of the serum vitamin B12 concentrations between metabolic and non-metabolic syndrome group, each group consisted of 26 subjects. According to NCEP ATPIII criteria, using statistical analysis by Mann–Whitney U test ($p<0.05$).

Table 4.3 Compare serum vitamin B12 concentrations between metabolic syndrome and non-metabolic syndrome

Parameter	Cutoffs	Number	Serum vitamin B12 (pg/ml) (mean ± SD)	p-value
NCEP ATP III criteria	Metabolic syndrome	26	693.02 ± 210.57	0.182
	Non-metabolic syndrome	26	634.75 ± 345.18	

Comparisons of the serum vitamin B12 concentrations between general characteristic profiles categorized into sex, age, physical activity, smoking behavior and drinking behavior in 52 subjects, using statistical analysis by Mann–Whitney U test ($p<0.05$).

Table 4.4 Compare serum vitamin B12 concentrations between general characteristic profiles

Parameter	Cutoffs	Number	Serum vitamin B12 (pg/ml) (mean ± SD)	p-value
Sex	Male	10	782.03 ± 238.73	0.063
	Female	42	635.76 ± 289.89	
Age	45-59	40	644.35 ± 290.36	0.287
	≥60	12	729.03 ± 265.79	
Physical activity	Active	37	656.28 ± 294.53	0.739
	Sedentary	15	682.66 ± 267.40	
Smoking	Yes	6	700.23 ± 196.92	0.492
	No	46	659.15 ± 295.46	
Alcohol	Yes	5	699.72 ± 214.27	0.587
	No	47	660.08 ± 292.69	

Comparisons of serum vitamin B12 concentrations between anthropometric parameters (body composition: BMI, WC, blood pressure) and biochemical parameters (laboratory: FBS, lipid profiles) in total 52 subjects. Data were analyzed using independent t-test^{a} and Mann–Whitney U test^{b}, as shown in Table 4.5

Table 4.5 Compare serum vitamin B12 concentrations between anthropometric and biochemical parameters

Parameter	Cutoffs	Number	Serum vitamin B12 (pg/ml) (mean ± SD)	p-value
BMI (kg/m^2)	Underweight (<18.5)	3	656.07 ± 565.55	0.406^{b}

Table 4.5 (continued)

Parameter	Cutoffs	Number	Serum vitamin B12 (pg/ml) (mean ± SD)	p-value
	Normal (18.5-22.9)	12	646.18 ± 243.88	
	Overweight (23-24.9)	12	686.58 ± 364.43	
	Obese I (25-29.9)	15	641.97 ± 263.22	
	Obese II (≥30)	10	693.15 ± 205.64	
WC (cm)	≥80 (F), ≥90 (M)	39	650.99 ± 241.69	0.891[a]
	<80 (F), <90 (M)	13	702.60 ± 396.40	
BP (mmHg)	≥130/85	28	654.28 ± 229.24	0.811[b]
	<130/85	24	675.10 ± 343.06	
FBS (mg/dL)	≥100	17	686.97 ± 199.55	0.364[b]
	<100	35	652.68 ± 319.96	
T.chol (mg/dL)	≥200	29	591.65 ± 256.08	0.044[b]
	<200	23	754.97 ± 297.95	
TG (mg/dL)	≥150	16	662.59 ± 233.33	0.983[a]
	<150	36	664.47 ± 307.74	
HDL (mg/dL)	<50 (F), <40 (M)	4	570.58 ± 90.70	0.559[a]
	≥50 (F), ≥40 (M)	48	671.67 ± 294.23	
LDL (mg/dL)	≥130	20	601.27 ± 272.05	0.164[b]
	<130	32	703.03 ± 289.47	

A significant difference was seen in comparison of serum vitamin B12 concentrations and total cholesterol (T.chol). Serum vitamin B12 concentrations in

subjects with high cholesterol (≥200 mg/dL) was significantly lower than in those with normal cholesterol (<200 mg/dL), using Mann–Whitney U test ($p<0.05$).

CHAPTER 5

DISCUSSION, CONCLUSION, AND SUGGESTIONS

5.1 Discussion

This research was aimed to compare serum vitamin B12 concentrations between metabolic syndrome and without metabolic syndrome which hypothesize that adults with metabolic syndrome have lower levels of serum vitamin B12 than those without metabolic syndrome. From the result of study, there was no significant difference between serum vitamin B12 level in two groups, serum vitamin B12 level in subjects with metabolic syndrome are slightly higher (693.02 ± 210.57) than non-metabolic syndrome (634.75 ± 345.18) at p-value = 0.182 (Table 4.3).

The results of the study above contradicted our hypothesis based on previous researches. A study reported by Yajnik mentioned that defects in one-carbon metabolism from low vitamin B12 concentrations might be the important part of adult metabolic diseases (Yajnik et al., 2008). A systematic review revealed that there were many reported literatures about the association between vitamin B12 deficiency and the development of atherosclerosis and other groups of coronary artery diseases (Chakraverty & Chakraborty, 2018). Furthermore, the study by Saraswathy was also suggested that deficiency of vitamin B12 could be disturbing two metabolic pathways which is at risk for cardio-vascular adversity leads to metabolic disorders (Saraswathy et al., 2018).

According to the supportive studies above, there were some issues that may prevent it from what this study were hypothesized and resulted in serum vitamin B12 level in metabolic syndrome subjects are found slightly higher than non-metabolic syndrome. For example: no dietary restrictions which may affect serum vitamin B12 concentration results, no data on participants' eating habits (except malnutrition, veterinarian, vegan that were collected), but not in detail or in terms of the type of food

they typically consume. No other diagnostic markers were obtained such as MMA and Hcy, which have been used by various similar studies to identify serum vitamin B12 concentration and deficiency, and were also specific and involved in cellular metabolism alongside vitamin B12. Furthermore, we did not compare the participants with or without metabolic syndrome in difference criteria, which could differed the results. This can be discussed further in the suggestions section.

However, another hypothesis of the study which anticipated in differences between serum vitamin B12 concentrations on metabolic syndrome parameters; including fasting blood sugar and lipid profiles. There was a significant difference in serum vitamin B12 concentrations and total cholesterol levels (Table 4.5). It was found that serum vitamin B12 in subjects with high cholesterol level (≥200 mg/dL), which mainly comes from patients with dyslipidemia, was lower than subjects with normal cholesterol level (<200 mg/dL). Similar to a study by Peter Lyon, which mentioned that the propionate catabolic pathway in mitochondria requires vitamin B12, as a cofactor to break down branched-chain amino acids (BCAAs), odd-chain fatty acids, and cholesterol for the tricarboxylic acid (TCA) cycle, thus decreased in vitamin B12 concentrations can reduce the propionate metabolism resulted in accumulation of BCAAs, odd-chain fatty acids, and cholesterol (Lyon et al., 2020).

Similar to the research by Boachie, which mentioned that deficiencies in vitamin B12, as methyl donors, are related to a higher risk of developing metabolic syndrome, obesity, and hepatic steatosis. In low vitamin B12 conditions, the expression of genes for the production of fatty acids, triglycerides, and cholesterol was upregulated, increases fatty acid synthesis, impaired fatty acid oxidation and mitochondrial respiration in hepatic cells, resulting in dysregulation of lipid metabolism in hepatocytes. It is referred to de novo hepatic lipogenesis and plays a role for dyslipidemia. Thus, if vitamin B12 has similar impacts on hepatocytes, this might be the explanation for the connection between low vitamin B12 and dyslipidemia in humans as well as the associated relationship seen in animal models (Boachie et al., 2021).

There was a no significant difference in serum vitamin B12 concentrations and the level fasting blood sugar, which contradict in our reference researches which suggested that subjects with high fasting blood sugar, specifically those with diabetes

mellitus who are usually treated with metformin, will have low or deficiency in serum vitamin B12 concentration compared to normal fasting blood sugar or non-DMs patients. According to the research by Fasipe, Long-term usage of metformin has been reported to be associated with intestinal malabsorption of vitamin B12, led to vitamin B12 deficiency (Fasipe et al., 2020). Similar to the study by Yamani which also found that deficiency of vitamin B12 resulted from prolonged metformin administration in patients with type 2 diabetes mellitus and routine screening of vitamin B12 before initiating metformin administration was recommended (Yamani et al., 2021).

Nevertheless, some research findings about relationship between vitamin B12 and fasting blood sugar contradict our hypothesis. For example, a study from Andres stated that diabetes mellitus patients might have elevated vitamin B12 due to increased oxidative stress, led to reduce conversion towards methionine, thus, vitamin B12 were not utilized related to defects in tissue uptake and action of vitamin B12 (Andrès et al., 2013). A study from Wei Li reported that patients with diabetes can have higher serum vitamin B12 levels than those with normal glycemic control, especially T2DM patients with wider glycemic fluctuations than in those with minor glycemic fluctuations, consequently, serum vitamin B12 levels could have an association with glycemic control in type 2 diabetes besides cause of metformin administration (Li et al., 2022). A study by Raizada found that metformin use was related to lower serum vitamin B12 levels, but after accounting for the period of time for diabetes, increasing duration of diabetes was linked to higher serum vitamin B12 levels. (Raizada et al., 2017).

Despite our primary hypothesis's outcome, which aim to compare serum vitamin B12 concentrations between metabolic and non-metabolic groups, found no significant difference. However, there was a significant difference between serum vitamin B12 concentrations and total cholesterol levels. Apparently, we found that serum vitamin B12 in subjects with high cholesterol levels was lower than subjects with normal cholesterol. The results are valuable and could benefit other researchers with an interest in this related topic of the study, providing evidences and details on the relationship between vitamin B12 and cholesterol regulations. Furthermore, the findings can be used to guide future studies into the possibility of treating or preventing dyslipidemia with the administration of vitamin B12 supplements, which could potentially prevent future metabolic syndrome.

5.2 Conclusion

5.2.1 Adults with metabolic syndrome and non-metabolic syndrome have no difference in serum vitamin B12 concentrations.

5.2.2 Serum vitamin B12 concentrations are significantly different between subjects with high cholesterol and normal cholesterol levels.

5.3 Suggestions

5.3.1 Considering Other Nutritional Assessment

Factors such as food intake should have been record more in details, especially type of food that contains nutritional source of vitamin B12 such as meat, eggs, dairy products, fish, and shellfish. In this study, solely data of malnutrition, veterinarian and vegan diet that were collected. Furthermore, nutritional assessment and other screening for vitamin B12 deficiency before data collecting is suggested, according to the American Society for Parenteral and Enteral Nutrition (ASPEN) and European Society for Clinical Nutrition and Metabolism (ESPEN). Nevertheless, we cannot limit our participant's nutrition activity since in each subject were not in under control which may differ the results of serum vitamin B12 concentrations.

5.3.2 Using Different Metabolic Syndrome Criteria

In this study, we did not compare the participants with or without metabolic syndrome in difference criteria, which are different in sensitivity, specificity and cut-off points of variables. Serum vitamin B12 concentrations may have a significant difference between the groups with and without metabolic syndrome as a result of methodological changes.

5.3.3 Further Study in Relationship Between Vitamin B12 and Cholesterol Regulation

The result of this study found that subjects with high cholesterol, who mainly consist of patients with dyslipidemia, had lower serum vitamin B12 concentrations than subjects with normal cholesterol. It could possibly be the result of different metabolisms

or factors that effect cholesterol levels and control. Further research on the connection between vitamin B12 and cholesterol regulation is suggested.

5.3.4 Considering Other Vitamin B12-Related Diagnostic Markers

In the present time, there is no gold standard for measuring vitamin B12 level. In cases of suspected deficiency, the standard first-line test is total serum vitamin B12 concentration using chemiluminescence. Despite the fact that serum vitamin B12 concentration may be the most common specific test ordered, they develop late after tissue levels have been depleted, thus, serum values can be normal despite tissue deficiency (Vanek et al., 2012). Other indicators of vitamin B12 deficiency including methylmalonic acid (MMA) and total homocysteine (Hcy) are examined if borderline value of serum vitamin B12 is detected (Romain et al., 2016). As a result of vitamin B12 deficiency, the conversion pathway in one-carbon metabolism declines, resulting in increased serum methylmalonic acid (MMA), while also disrupts the methylation process which leads to accumulation of serum homocysteine (Hcy) (Davis et al., 2013). Hence, serum MMA and Hcy are considered collecting as other diagnostic markers together with serum vitamin B12 concentration in this study.

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APPENDICES

APPENDIX A

ETHIC APPROVAL

The Mae Fah Luang University Ethics Committee on Human Research
333 Moo 1, Thasud, Muang, ChiangRai 57100
Tel: (053) 917-170 to 71, (053) 916-551 Fax: (053) 917-170 E-mail: rec.human@mfu.ac.th

CERTIFICATE OF APPROVAL

COA: 226/2023 **Protocol No: EC 23185-20**

Title: THE COMPARISON BETWEEN SERUM VITAMIN B12 CONCENTRATIONS IN ADULTS WITH AND WITHOUT METABOLIC SYNDROME

Principal investigator: Ajirapa Bussaracom, M.D.

School: Anti Aging and Regenerative Medicine

Funding support: Personal Budget

Approval:

1) Research protocol — Version 2 Date November 8, 2023
2) Information sheet and informed consent documents — Version 2 Date November 8, 2023
3) Questionnaires — Version 2 Date November 8, 2023
4) Case record forms — Version 1 Date August 28, 2023
5) Principal investigator and Co-investigators
   - Ajirapa Bussaracom, M.D. - Vitoon Jularattanaporn, M.D., Ph.D.

The aforementioned documents have been reviewed and approved by the Mae Fah Luang University Ethics Committee on Human Research in compliance with international guidelines such as Declaration of Helsinki, the Belmont Report, CIOMS Guidelines and the International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use - Good Clinical Practice (ICH GCP)

Date of Approval: November 21, 2023

Date of Expiration: November 20, 2024

Frequency of Continuing Review: 1 year

(Assoc. Prof., Maj. Gen. Sangkae Chamnanvanakij, M.D.)
Chairperson of the Mae Fah Luang Ethics Committee on Human Research

The Mae Fah Luang University Ethics Committee on Human Research
333 Moo 1, Thasud, Muang, ChiangRai 57100
Tel: (053) 917-170 to 71, (053) 916-551 Fax: (053) 917-170 E-mail: rec.human@mfu.ac.th

หนังสือรับรองด้านจริยธรรมการวิจัย

COA: 226/2023 รหัสโครงการวิจัย: EC 23185-20

ชื่อโครงการวิจัย : การวิจัยเปรียบเทียบระดับค่าความเข้มข้นของวิตามินบี 12 ในเลือดของผู้ที่มีภาวะเมแทบอลิกซินโดรม และไม่มีภาวะเมแทบอลิกซินโดรม

ชื่อผู้วิจัยหลัก: แพทย์หญิง อจิรภาส์ บุษราคำ

สำนักวิชา: เวชศาสตร์ชะลอวัยและฟื้นฟูสุขภาพ

ผู้สนับสนุนทุนวิจัย: ทุนส่วนตัว

การรับรอง :

(1) โครงร่างการวิจัย ฉบับที่ 2 วันที่ 8 พฤศจิกายน 2566
(2) เอกสารข้อมูลและขอความยินยอมเข้าร่วมการวิจัย ฉบับที่ 2 วันที่ 8 พฤศจิกายน 2566
(3) แบบสอบถาม ฉบับที่ 2 วันที่ 8 พฤศจิกายน 2566
(4) แบบบันทึกข้อมูล ฉบับที่ 1 วันที่ 28 กันยายน 2566
(5) ผู้วิจัย และผู้วิจัยร่วม
- แพทย์หญิง อจิรภาส์ บุษราคำ - อาจารย์ ดร. นพ. วิทูร จุลรัตนาภรณ์

ขอรับรองว่าโครงการดังกล่าวข้างต้นได้ผ่านการพิจารณารับรองจากคณะกรรมการจริยธรรมการวิจัยในมนุษย์ มหาวิทยาลัยแม่ฟ้าหลวง ว่าสอดคล้องกับแนวทางจริยธรรมสากล ได้แก่ ปฏิญญาเฮลซิงกิ (Declaration of Helsinki) รายงานเบลมองต์ (Belmont Report) แนวทางจริยธรรมสากลสำหรับการวิจัยในมนุษย์ของสภาองค์การสากลด้านวิทยาศาสตร์การแพทย์ (CIOMS) และแนวทางการปฏิบัติการวิจัยที่ดี (ICH GCP)

วันที่รับรองด้านจริยธรรมของโครงร่างการวิจัย: 21 พฤศจิกายน 2566
วันสิ้นสุดการรับรอง: 20 พฤศจิกายน 2567
ความถี่ของการส่งรายงานความก้าวหน้าของการวิจัย: 1 ปี

ลงนาม .....

(รองศาสตราจารย์ พลตรีหญิง แพทย์หญิง แสงแข ชำนาญวนกิจ)
ประธานคณะกรรมการจริยธรรมการวิจัยในมนุษย์ มหาวิทยาลัยแม่ฟ้าหลวง

APPENDIX B

INFORMED CONSENT FORM

หนังสือแสดงความยินยอมเข้าร่วมการวิจัย

ข้าพเจ้า _____ ตัดสินใจเข้าร่วมการวิจัยเรื่อง การเปรียบเทียบระดับค่าความเข้มข้นของวิตามินบี 12 ในเลือด ของผู้ที่มีกลุ่มอาการเมตาบอลิกและไม่มีกลุ่มอาการเมตาบอลิก ซึ่งข้าพเจ้าได้รับข้อมูลและคำอธิบายเกี่ยวกับการวิจัยนี้แล้ว และได้มีโอกาสซักถามและได้รับคำตอบเป็นที่พอใจแล้ว ข้าพเจ้ามีเวลาเพียงพอในการอ่านและทำความเข้าใจข้อมูลในเอกสารให้ข้อมูลสำหรับผู้เข้าร่วมการวิจัยอย่างถี่ถ้วน และได้รับเวลาเพียงพอในการตัดสินใจว่าจะเข้าร่วมการวิจัยนี้

ข้าพเจ้ารับทราบว่าข้าพเจ้าสามารถปฏิเสธการเข้าร่วมการวิจัยนี้ได้โดยอิสระ และระหว่างการเข้าร่วมการวิจัย ข้าพเจ้ายังสามารถถอนตัวออกจากการวิจัยได้ทุกเมื่อ โดยไม่ส่งผลกระทบต่อการดูแลรักษา หรือสิทธิที่ข้าพเจ้าพึงมี

โดยการลงนามนี้ ข้าพเจ้าไม่ได้สละสิทธิใด ๆ ที่ข้าพเจ้าพึงมีตามกฎหมาย และหลังจากลงนามแล้ว ข้าพเจ้าจะได้รับเอกสารข้อมูลและขอความยินยอมไว้จำนวน 1 ชุด

ลายมือชื่อผู้เข้าร่วมการวิจัย _____ วัน-เดือน-ปี _____

(_____)

..... (กรณีที่ผู้เข้าร่วมการวิจัยอ่านหนังสือไม่ออกแต่ฟังเข้าใจ) .....

ข้าพเจ้าไม่สามารถอ่านหนังสือได้ แต่ผู้วิจัยได้อ่านข้อความในเอกสารข้อมูลและขอความยินยอมนี้ให้แก่ข้าพเจ้าฟังจนเข้าใจดีแล้ว ข้าพเจ้าจึงลงนามหรือพิมพ์ลายนิ้วมือของข้าพเจ้าในหนังสือนี้ด้วยความสมัครใจ

ลงนาม/พิมพ์ลายนิ้วมือผู้เข้าร่วมการวิจัย _____ วัน-เดือน-ปี _____

(_____)

ลายมือชื่อผู้ขอความยินยอม _____ วัน-เดือน-ปี _____

(_____)

THE MAE FAH LUANG UNIVERSITY
ETHICS COMMITTEE ON HUMAN RESEARCH
21 NOV 2023
APPROVED

คำรับรองของพยานผู้ไม่มีส่วนได้เสียกับการวิจัย (กรณีที่ผู้เข้าร่วมการวิจัยอ่านหนังสือไม่ออกแต่ฟังเข้าใจ)

ข้าพเจ้าได้อยู่ร่วมในกระบวนการขอความยินยอมและยืนยันว่า ผู้ขอความยินยอมได้อ่าน/อธิบายเอกสารข้อมูลให้แก่ _____ ซึ่งผู้มีชื่อข้างต้นมีโอกาสซักถามข้อสงสัยต่าง ๆ และได้ให้ความยินยอมเข้าร่วมการวิจัยโดยอิสระ หลังจากรับทราบข้อมูลที่มีอยู่ตรงตามที่ปรากฏในเอกสารนี้แล้ว

ลายมือชื่อพยาน _____ วัน-เดือน-ปี _____

(_____)

กรณี อาสาสมัครที่เป็นเด็ก อายุน้อยกว่า 18 ปี หรือเป็นบุคคลที่มีปัญหาทางจิตหรือสติปัญญา ต้องได้รับการปกป้องคุ้มครองเป็นพิเศษ

ข้าพเจ้าในฐานะ _____ กับผู้เข้าร่วมการวิจัย ได้อ่านข้อความข้างต้นและมีความเข้าใจดีทุกประการแล้ว ยินยอมให้ ด.ช./ด.ญ./นาย/นาง/นางสาว _____ เข้าร่วมการวิจัยด้วยความสมัครใจ จึงได้ลงนามในเอกสารใบยินยอมนี้

ลายมือชื่อผู้แทนโดยชอบธรรม/ผู้ปกครอง_____ วัน-เดือน-ปี _____

(_____)

APPENDIX C

QUESTIONNAIRE

แบบสอบถามการวิจัย

อายุ ____ ปี

เพศ ☐ ชาย ☐ หญิง

โรคประจำตัว

☐ ความดันโลหิตสูง
☐ ไขมันในเลือดสูง
☐ เบาหวาน
☐ ภาวะอ้วน
☐ โรคไทรอยด์
☐ โรคกระดูกและข้อ (OA, gout, spondylosis, neuropathy)
☐ โรคทางเดินอาหาร (chronic dyspepsia, GERD)
☐ โรคทางเดินหายใจ (AR, asthma, COPD)
☐ โรคทางระบบประสาทและหลอดเลือดสมอง ระบุ (_____)
☐ โรคอื่นๆ ระบุ_____

☐ โรคไตและทางเดินปัสสาวะ (CKD, ESRD, KUB stone)
☐ โรคตับ (HBV, HCV, cirrhosis)
☐ โรคทางภูมิคุ้มกัน (SLE, psoriasis, rheumatoid)
☐ การติดเชื้อเรื้อรัง (HIV, TB, syphilis)
☐ โรคมะเร็ง ระบุ (_____)

☐ ปฏิเสธโรคประจำตัว

ยาที่ใช้ประจำ _____

_____

☐ ปฏิเสธยาที่ใช้ประจำ

ประวัติการผ่าตัด _____

_____

☐ ปฏิเสธประวัติการผ่าตัด

เงื่อนไขทางการแพทย์ที่เกี่ยวข้องกับ วิตามินบี 12

☐ อยู่ระหว่างการใช้วิตามินบีเพื่อเป็นอาหารเสริมหรือรักษาโรค
☐ มีภาวะโลหิตจาง/ซีด ระบุ _____ (ธาลัสซีเมีย, ขาดธาตุเหล็ก)
☐ มังสวิรัติ / มังสวิรัติ (vegetarian/vegan) หรือ ภาวะขาดสารอาหาร

☐ ปฏิเสธเงื่อนไขทางการแพทย์ดังกล่าว

อาชีพ

☐ เกษตรกร
☐ ข้าราชการ
☐ พนักงานบริษัทเอกชน
☐ อาชีพอิสระ
☐ ธุรกิจส่วนตัว
☐ อื่นๆ ระบุ _____
☐ ไม่ประกอบอาชีพ / เกษียณอายุ

การออกกำลังกาย

☐ คล่องแคล่ว / กระตือรือร้น (ออกกำลังกาย, ทำงานอย่างหนักทางร่างกาย)
☐ นั่งเฉยๆ / ไม่ค่อยขยับ (ไม่ค่อยออกกำลังกาย, ทำงานอยู่กับที่)

การสูบบุหรี่

☐ มีประวัติสูบบุหรี่ต่อเนื่อง มากกว่า 15 ปี
☐ มีประวัติสูบบุหรี่ต่อเนื่อง น้อยกว่า 15 ปี
☐ เลิกสูบบุหรี่ (ตั้งแต่ _____)
☐ ไม่สูบบุหรี่ / ไม่เคยสูบบุหรี่

การดื่มเครื่องดื่มแอลกอฮอล์

☐ ประวัติการดื่มแอลกอฮอล์ต่อเนื่อง _____ ปี
☐ การดื่มเพื่อสังคม / นานๆครั้ง
☐ เลิกเครื่องดื่มแอลกอฮอล์ (ตั้งแต่ _____)
☐ ไม่ดื่มแอลกอฮอล์ / ไม่เคยดื่ม

APPENDIX D

CASE RECORD FORM

Case record form

Date of study visit : ☐☐/☐☐/☐☐

1. Subject identification (ID)		
1.1 Subject group (A : Metabolic syndrome , B : non-Metabolic syndrome)	☐ A	☐ B
1.2 Number : (01-25)		
1.3 Subject ID : (ex. A01-A25, B01-B25)		

2. Screening Form		
3.1 Inclusion checklist		
a. Thai participants aged between 40 and 60 years old.	☐ Yes	☐ No
b. Participants with metabolic syndrome	☐ Yes	☐ No
c. Participants without metabolic syndrome	☐ Yes	☐ No
d. Participants accepted to examine blood samples.	☐ Yes	☐ No
e. Participants are willing to participate in the study.	☐ Yes	☐ No
3.2 Exclusion checklist		
a. Participants who have a history of vitamin B supplement or medical treatment involved vitamin B within 12 months prior to study.	☐ Yes	☐ No
b. Participants who have any medical or surgical conditions which could reduced vitamin B12 intake or absorption.	☐ Yes	☐ No
c. Participants who have any anemic disease such as pernicious anemia, hemolytic anemia, or anemia of chronic diseases.	☐ Yes	☐ No
d. Participants who are vegetarian/vegan, under malnutrition, or have a history of chronic alcohol consumption.	☐ Yes	☐ No
e. Participants who had a history of autoimmune disease, malignancy, chronic infection, or chronic liver or kidney disease.	☐ Yes	☐ No
2.3 Medical history 2.3.1 Underlying diseases ☐ HT ☐ chronic kidney disease (CKD,ESRD) ☐ DLP ☐ chronic liver disease (HBV, HCV, cirrhosis) ☐ DM type 2 ☐ autoimmune disease (SLE, psoriasis, rheumatoid) ☐ central obesity ☐ chronic infection (HIV, TB, syphilis) ☐ thyroid disease ☐ malignancy (CA_____) ☐ musculoskeletal disease (OA, gout, spondylosis, neuropathy) ☐ gastrointestinal disease (chronic dyspepsia, GERD) ☐ respiratory disease (AR, asthma, COPD) ☐ others _____ ☐ no U/D		
2.3.2 Medical conditions involving vitamin B12 concentrations		
a. vitamin B supplement or treatment (ex. vitamin B complex, B1-6-12)	☐ Yes	☐ No
b. anemic diseases	☐ Yes	☐ No
c. vegetarian/vegan, under malnutrition	☐ Yes	☐ No

2.3.3 Current medication		
a. colchicine	☐ Yes	☐ No
b. chloramphenicol	☐ Yes	☐ No
c. histamine antagonists	☐ Yes	☐ No
d. metformin	☐ Yes	☐ No
e. proton pump inhibitors (PPI)	☐ Yes	☐ No
f. corticosteroids or chemotherapy medications	☐ Yes	☐ No

2.4 Other Data

2.4.1 Occupation

- ☐ agricultural workers/farmers
- ☐ government employees
- ☐ company employees
- ☐ self-employed
- ☐ business owners
- ☐ students
- ☐ others _____
- ☐ none / retired persons

2.4.2 Physical activity

- ☐ active (exercise, physically strenuous work)
- ☐ sedentary/ inactive (sitting and lying down, very little to no exercise)

2.4.3 Smoking

- ☐ history of active smoking > 15 years
- ☐ history of active smoking < 15 years
- ☐ quit smoking (since _____)
- ☐ non-smokers / never smoke

2.4.5 Alcohol consumption

- ☐ history of chronic alcohol consumption _____ years
  - ☐ social drinking
  - ☐ quit drinking (since _____)
- ☐ no drinking / never drink

3. Baseline assessment

3.1 Physical examination : Anthropometric measurements (Body composition)

3.1.1 Weight (kg) _____

3.1.2 Height (cm) _____

[calculated BMI (kg/m^2) _____]

- ☐ overweight defined as BMI 23–24.9 kg/m2
- ☐ obese defined as BMI ≥ 25 kg/m2

3.1.3 Waist circumference: WC (cm) _____

- ☐ obesity defined as WC ≥ 90 cm for men , WC ≥ 80 cm for women

3.1.4 Hip circumference: HC (cm) _____

[calculated Waist hip ratio: WHR _____]

- ☐ high WHR was defined as ≥ 0.90 for men ,0.80 for women

3.1.5 Blood pressure (mmHg) _____ (hypertension: >140/90)

3.2 Laboratory investigation : Biochemical data

3.2.1 Blood for metabolic diseases

1.1 Serum fasting glucose (FBS) _____ (70 – 100 mg/dL)

100 - 125 mg/dL: prediabetes

> 126 mg/dL: diabetes

Lipid profiles

1.2 Total cholesterol (TC) _____ (high TC: > 200 mg/dL)

1.3 Triglycerides (TG) _____ (high TG: ≥ 150 mg/dL)

1.4 High-density lipoprotein cholesterol (HDL-C) _____ (low HDL: < 40 mg/dL)

1.5 Low-density lipoprotein cholesterol (LDL-C) _____ (high LDL: >130 mg/dL)

2. Serum vitamin B12 concentrations (VitB12)_____ (197-771 pg/ml)

❑ vitamin B-12 deficiency defined as ≤ 220 pg/ml

THE MAE FAH LUANG UNIVERSITY
ETHICS COMMITTEE ON HUMAN RESEARCH

2 1 NOV 2023

APPROVED

CURRICULUM VITAE

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NAME Ajirapa Bussaracom

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