CHAPTER 1

Introduction

Obesity is one of the world's leading health problems. It reduces the quality of life and health for people affected by it. It has been directly associated with 3.4 million deaths each year [1]. In 2013, 37% of world's adult population was overweight or obese, with 65% of the obese population coming from developing countries. Furthermore, both the number of obese people and the overweight population are increasing at an alarming rate [2]. Reports from 2010 have shown that the prevalence of overweight people in Thailand was 28.1%, and the prevalence of obesity was 6.9% Young adulthood is a transitioning period that takes place between adolescence and adulthood. This period occurs during the ages of 16-30 years. This age group is affected by various influences as they engage with the ever-changing environment. The social, biological, and psychological influences all affect the individual well into adulthood. These factors can cause young adults to be at a higher risk of developing problematic lifestyles, especially for university students. Increasingly, evidence suggest that young adults have higher risks for developing unhealthy lifestyles, may excessively consume food and sugary drinks, and can also make risky decisions regarding their own lives. Any behavior changes occurring in this age group can be either positive or negative, and tends to continue well into the later years of life. This age group also is at a higher risk for gaining weight at 1 kg/year [3], with a 2 fold prevalence of obesity occurring during this period when compared to younger ages [4]. These statistics are consistent with previous studies of BMI trends taking place in Thailand. This pattern of weight increase was consistently true for both sexes and for all ages despite socioeconomic status. The number of obese people in the Thai population (>30 kg/m^2) aged between 25-35 years old has almost doubled [5]. Despite recognizing the contributing factors leading to widespread obesity, very little research has been done

on, and very little attention has been paid to young adults in this age group. That is, until very recently.

Obesity was known to be one of the health risk factors that lead to many obesity-related disease, such as dyslipidemia, cardiovascular disease, insulin resistance, hypertension and non-alcoholic fatty liver disease (NAFLD). NAFLD is one of the most common chronic liver disease and is increasing in number at alarming rate.

The prevalence of NAFLD is Asia is around 27.4%, which is quite similar to the levels found in western countries [6]. Among NAFLD patients, 51% are obese, and 82% of non-alcoholic steatohepatitis (NASH) patients are considered obese, as well. The number of NAFLD patients have been increasing, as this tendency mirrors the same increases taking place globally [7].

NAFLD in young adults is a topic that has received little recognition, despite the fact that this age group is most likely to gain weight and develop obesity from poor diet and risky lifestyles as they are transitioning into adulthood [3]. The prevalence of NAFLD in young adults has been increasing almost 2.5 times over the past 30 years, with half of all morbidly obese young adults having NAFLD [8]. However, despite the growing public health concerns about obesity and NAFLD in young adults, necessary infromation addressing the effects of obesity and NAFLD pathogenesis in this age group is in short supply, and there is an urgent need for better understandingof its effects and mechanisms [4, 9].

There are several factors that contribute to the accumulation of liver fat. The main causes are obesity, sedentary lifestyle, and excessive sugar intake. These dominant factors lead to the accumulation of lipids in the body. Lipids are important biomolecules and have several roles in the body. First, they act as an energy source. Secondly, they are a component of cell membranes. They are also they are involved in the synthesis of steroid hormones. However, excessive accumulation of body fat can lead to weight gain and obesity. Gaining weight is primarily caused by long term energy imbalances, high caloric intake, and sedentary lifestyle. Body lipids are mostly stored as triglycerides from adipose tissue. Whenever adipose cells are unable to proliferate and differentiate, triglycerides from excessive lipid supply, leads to adipose tissue dysfunction and causes a spill-over

of lipids into non-adipose tissue that normally have a limited capacity to stored fat; such as in the musculoskeletal muscles, pancreas, cardiac muscles, and the liver. The fat deposited in these non-adipose tissues is called ectopic fat [10]. Previous research has established that ectopic fat in the liver and skeleton muscle are closely related with insulin resistance and metabolic syndromes such as obesity, high blood pressure, high blood sugar, and dyslipidemia.

A number of studies have found that weight loss and exercise promote the reduction of liver fat content (LFC) and liver insulin resistance [11-14]. At the same time, liver fat content also can be influenced by internal factors such as sex, age, race, and genetics [15]. Previous studies have found that men have a higher prevalence of NAFLD than women. However, the risk of NAFLD in women increases with age, especially in postmenopausal women [16, 17]. Moreover, it has now been demonstrated that there is a linear correlation between age and NAFLD risk [18]. More recent evidence shows a higher risk of NAFLD for anyone that has a family member with NALFD. Among people having NAFLD, 39% have inherited this condition independent of age, sex, BMI, and ethnicity [19].

There is a considerable body of literature on the multifactor effects of obese that lead to fatty liver, dyslipidemia, and insulin resistance. Aside from ectopic fat, dysfunction of adipose tissue has also been identified as one of the factors causing insulin resistance by secretion of pro-inflammatory cytokines that activate immune cells such as macrophages. As the macrophages react to chronic and low-level inflammation, the M1-like macrophages secrete various cytokines that may impair organs and whole-body insulin sensitivity [20]. However, NAFLD does not directly cause the insulin resistance, but rather is considered to be a consequence or a second effect from hyperlipidemia and insulin resistance [21].

It is well-established that being overweight and obese is associated with dyslipidemia. Dyslipidemia results from a composition of high blood triglyceride levels, high levels of low density lipoprotein (LDL), and low levels of high density lipoprotein (HDL) [22, 23]. It is evaluated by determining the free fatty acid levels in plasma and the liver, especially with regard to the levels of saturated fatty acids that have been found to be associated with obesity, insulin resistance [24] and fatty liver [25]. Insulin resistant patients have increased adipose tissue lipolysis. Previous studies have found that visceral fat lipolysis

releases free fatty acids (FFA) into portal veins, with over 30% of FFA delivered to the liver in obese subjects. Conversely, only 5 – 10 % of FFA is delivered to the liver in normal weight subjects [26].

Previous studies have reported positive correlation of BMI and lipid accumulation in the liver, a higher risk of NAFLD, cirrhosis [27, 28] and dyslipidemia [29]. A fatty liver leads to variety of chronic liver diseases, ranging from simple fat deposits in the liver occurring without alcohol consumption; or NAFLD, which can progress from prolonging inflammation to NASH, and eventually causes cell death to form scar tissue on the liver called fibrosis. Advanced fibrogenesis leads to cirrhosis with extensive fibrosis, impaired liver function, regenerative nodules and liver failure. Cirrhosis is also related to an increased risk of hepatocellular carcinoma.

Based on understanding of the progression of disease from NAFLD, there is urgent need of NAFLD detection before it progresses to NASH. Currently, liver biopsies are the gold standard of NAFLD assessment in clinical settings [30]. Biopsies are invasive techniques in which small samples of liver tissue are removed, but it also has several limitations. First, a liver biopsy is a small proportion of the whole liver and may not represent the whole liver histology. Depending on the site of the needle puncture, effected sites in the liver may not be found . Second, it is an invasive technique, not suitable for screening tests and frequent follow ups [31]. There are other methods for assessing LFC available for use. These include ultrasound, computed tomography (CT), and proton magnetic resonance spectroscopy (^{1}H MRS). Ultrasound is proven to be cost-effective, has high accessibility, and is not time consuming. However, diagnosis results and grading is highly dependent on the reader and radiologist. CT is accurate for diagnosis of moderate to severe steatosis but is not accurate for mild steatosis and exposes patients to radiation. Therefore, CT is not suitable for longitudinal follow-ups [25, 30]. ^{1}H MRS is a well-established non-invasive technique utilized for assessing liver metabolites in various organs using magnetic resonance imaging (MRI) machines.

The biochemical data obtained from ^{1}H MRS is a spectrum. The spectra contain series of peaks at various frequencies. Each metabolite has different properties and chemical environments that reveal unique frequency shift. These unique frequency shifts called chemical shifts that use to identify metabolites. The integration of areas under the peak

of represent the quantity of that peak's metabolite [32]. ^{1}H MRS uses similar principles as that of MRI, utilizing spin properties of nuclear to generate signals. The most common nuclear that is used for metabolite in-vivo studies is proton (^{1}H), which is the most abundant molecule found in the body.

^{1}H MRS is used in liver fat studies. The dominate peaks of ^{1}H MRS obtained from the liver was found at 0.9, 1.3, 2.1 and 2.75 ppm [33, 34]. Previous research has established that ^{1}H MRS for LFC quantification is consistent with biopsy methods, and is able to determine the severity of liver damage and hepatic steatosis progression. Moreover, it was also known for being highly accurate for quantifying LFC, is suitable for longitudinal follow-up with high sensitivity of 72.7 – 88.5 %, and shows high specificity of 92.0 – 95.7 % [35, 36].

Another technique that uses the same principles as MRI and ^{1}H MRS is nuclear magnetic resonance spectroscopy (NMR). NMR is one of the most popular techniques for in-vitro biomarker study. NMR is able to provide chemical information about the compounds, molecular interaction, and is often used in molecular formation studies. NMR is also used in medical research, such as for research done on serum metabolites, as it is able to give information about many metabolites in serum in one exam, including the lipids and the levels of very low density lipoproteins (VLDL), without the need of separate biochemical tests [37].

Metabolites are an intermediate byproducts of body metabolism occurring in such compounds as amino acids, nucleotides, sugars, and fatty acids. Changes in the numbers of metabolites reflect the changes in cellular activity, and allows prediction of disease development and progression. Therefore, NMR is a suitable technique for metabolite profile studies that reflect the health status of an individual. Blood tests are used for diagnosing diseases, can assess the functioning of organs, and can reflect health status. NMR has been used to assess the various serum metabolomics in studies done on lipids, metabolic syndromes, and liver diseases. It is also used in metabolite profile studies in diabetes patients [38], to study the effects of exercise on metabolites [39], effect of saturated fat and non-saturated fat in diet on the serum metabolites, accumulations of liver and muscle lipid [40], and for serum studies in hepatitis c patients to detect alterations of metabolites that reflect disease progression that may permit early diagnosis of liver cancer

[41]. From all these studies, it has been shown that the NMR technique is a powerful technique for gaining valuable knowledge in many areas.

As discussed above, blood tests, weight gain, chemical processes occurring in the liver, and lipid accumulation are all associated with lipid metabolism, pathophysiological changes, and the development and progression of fatty livers. The mechanism behind NAFLD pathogenesis is not yet fully understood. NAFLD in young adults is a topic that has received little recognition, yet this age group is the most likely one to gain weight and develop obesity from diet and life style as they are transitioning into adulthood. This is particularly true when considering the prevalence of overweight and obese people living in developing countries [3]. The numbers of the obese have almost doubled among young adults compared to younger ages, and the obesity will likely persist into older age. Understanding the development of obesity and pathogenesis in this age group is fundamentally important for developing knowledge about prevention [4]. As far as we know, there's no study to date that has investigated the effects of obesity on liver fat content (LFC) by ^{1}H MRS. Moreover, most studies were done on subjects with diseases such as diabetes, cancer, and chronic diseases. This focus has led to uncertainty about the health effects from being overweight and any pathogenesis taking place in healthy subjects.

The aim of this study is to assess the association between liver fat content (LFC) and the effects of being overweight on liver fat content (LFC) and serum metabolites using ^{1}H MRS and NMR technique. Liver metabolite and serum levels of 30 healthy young adults subjects (ages 19-35 years old) having BMIs in the normal range of18.5-24.9 kg/m^2, and 30 healthy young adults subjects ages 19-35 years old with BMIs of more than 25 kg/m^2 were selected for this study. The ^{1}H MRS data of liver was obtained from the right lobe of the liver (Couinaud lobe segment V-VIII) [42] with metabolite serum studies done on NMR machines combined with laboratory blood tests.

1.1 Objective

The main aim of this study is to assess and study the relationship of LFC and serum levels of healthy overweight persons using ^{1}H MRS and NMR.